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PROCEEDINGS OF THE FIFTY-FOURTH ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1938

The fifty-fourth annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington, D. C., November 14, 15, and 16, 1938.

The meeting was called to order by the president, H. R. Kraybill, Purdue University, Lafayette, Ind., on the morning of November 14, at 10:30 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING NOVEMBER, 1939

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PERMANENT COMMITTEES

Recommendations of Referees

(Figures in parentheses refer to year in which appointment expires.)

H. A. LEPPER (U. S. Food and Drug Administration, Washington, D. C.), *Chairman*
SUBCOMMITTEE A: G. E. Grattan (1940), (Department of Agriculture, Ottawa, Can.), *Chairman;* H. A. Halvorson (1942) and E. L. Griffin (1944). [Standard solutions (silver nitrate and thiocyanate, iodine and thiosulfate, sulfuric acid, potassium permanganate); insecticides, fungicides, and caustic poisons (fluorine compounds; pyrethrins, derris, and cubé; naphthalene in poultry lice products); soils and liming materials (hydrogen-ion concentration—soils of arid and semi-arid regions and soils of humid regions; liming materials, less common metals in soils, selenium); feeding stuffs (sampling, ash, mineral mixed feeds—calcium and iodine; moisture, lactose in mixed feeds, hydrocyanic acid in glucoside-bearing materials, biological methods for determination of vitamin D carriers, biological methods for vitamin B complexes, technic and details of biological methods—vitamin D carriers, carotene, manganese, adulteration of condensed milk products and of cod-liver oil, fat in fish meal); fertilizers (phosphoric acid, nitrogen, magnesium and manganese, acid- and base-forming qual-

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ity, potash; calcium, sulfur, copper, and zinc); plants (less common metals, carbohydrates, inulin, hydrocyanic acid, forms of nitrogen, sodium and potassium); lignin, enzymes (papain); paints, paint materials and varnishes (accelerated testing of paints, varnishes); vitamins (vitamin A, vitamin D, vitamin K, riboflavin); leathers and tanning materials, disinfectants.]

SUBCOMMITTEE B: H. J. FISHER (1940), (Agricultural Experiment Station, New Haven, Conn., *Chairman;* A. E. PAUL (1942) and W. F. REINDOLLAR (1944). [Naval stores; radioactivity (quantum counter, gamma ray scope); cosmetics; drugs (microchemical tests for alkaloids, microchemical tests for synthetics, daphnia methods, ergot alkaloids, guaiacol in mixtures, biological testing, iodine ointment, elixir of terpin hydrate and codeine, aminopyrine and phenobarbital in mixtures, ointment of yellow mercuric oxide, rhubarb and rhaponticum, theophylline sodium salicylate, ephedrine in jellies, arecoline hydrobromide, separation of acetanilid and salol; acetylsalicylic acid, acetophenetidin and salol; benzedrine, plasmochine, physostigmine salicylate, ipecac, opium powder (Dover's powder), pepsin, hydroxyquinoline sulfate nicotinic acid, purification of caffeine).

SUBCOMMITTEE C: G. G. FRARY (1940), (State Chemical Laboratory, Vermillion, S. D.), *Chairman;* W. B. WHITE (1942) and J. O. CLARKE (1944). [Dairy products (butter, cheese—isolation of fat, malted milk—fat, casein, dried milk—lactic acid, lactose in milk, mold in butter, decomposition, neutralizers, difference between dairy products made from cows' milk and those made from milk of other animals, frozen desserts, tests for pasteurization—milk and cream, butter); oils, fats and waxes (refractometric determination of oil in seeds, thiocyanogen number, Polenski number); eggs and egg products (unsaponifiable constituents and fat, detection of decomposition—glycerol and sugar); metals in foods (arsenic and antimony, copper, zinc, fluorine, lead, mercury, selenium, fumigation residues in foods); canned foods (tomato products); meats and meat products; spices and condiments (salad dressings, vinegar, volatile constituents); gums in foods (starchy foods); microbiological methods (canned fish products, canned meats, canned vegetables, canned tomato products, sugar, eggs and egg products); fish and other marine products (solids and fats); nuts and nut products; coffee and tea.]

SUBCOMMITTEE D: W. C. JONES (1940), (Department of Agriculture, Richmond, Va.), *Chairman;* J. W. Sale (1942) and J. A. LeClerc (1944). [Sugars and sugar products (acetyl-methyl carbinol and diacetyl in food products, sucrose and ash in molasses, honey, refractive indices of sugar solutions, maple products; drying, densimetric, and refractometric methods; polariscopic methods, chemical methods for reducing sugars, sugars in molasses); waters, brine, and salt (effervescent salts); alcoholic beverages (diastatic activity of malt, proteolytic activity of malt, malt extract in malt, malt adjuncts, beer, CO_2 in beer, heavy metals in beer, total sulfur in wine, volatile acids in wine, volatile acids in distilled spirits, SO_2 in wine and beer, aldehydes in whiskey and other potable spirits, detection of adulteration of distilled spirits, wood alcohol in brandy, cordials and liqueurs); food preservatives (saccharine, benzoate of soda); coloring matters in foods; fruits and fruit products (electrometric titration of acids; malic, isocitric and lactic acids; polariscopic methods for, and ash in, jams, jellies, and preserves; P_2O_5 in jams, jellies, and other fruit products); cacao products; cereal foods (ash in flour, macaroni products, and baked products; H-ion concentration of flour, starch in flour, acidity in flour, sugar in flour, baking test for soft wheat flour, flour-bleaching chemicals, CO_2 in self-rising flour, milk solids in milk bread, cold water extract flour, ergot in flour, proteo-

lytic enzymes, color in flour, soya flour in foods, whole wheat flour, phosphated flour, sterols, corn products, oat products, rye and buckwheat, barley and rice, baked products other than bread); microchemical methods; flavors and non-alcoholic beverages (organic solvents in flavors); baking powder—tartrates.]

Committee to Cooperate with Other Committees on Food Definitions

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STANDARD SOLUTIONS:
  General referee: R. L. Vandaveer, Food and Drug Adm., New Orleans, La.

  SILVER NITRATE AND THIOCYANATE:
    Associate referee: R. L. Vandaveer

  IODINE AND THIOSULFATE:
    Associate referee: K. L. Milstead, Food and Drug Adm., Chicago, Ill.

  SULFURIC ACID:
    Associate referee: W. H. King, Food and Drug Adm., New Orleans, La.

  POTASSIUM PERMANGANATE:
    Associate referee: G. M. Johnson, Food and Drug Adm., St. Louis, Mo.

INSECTICIDES, FUNGICIDES, AND CAUSTIC POISONS:
  General referee: J. J. T. Graham, Food and Drug Adm., Washington, D. C.

  PYRETHRINS, DERRIS, AND CUBÉ:
    Associate referee: J. J. T. Graham.

  FLUORINE COMPOUNDS:
    Associate referee: C. G. Donovan, Bureau of Entomology and Plant Quarantine, Washington, D. C.

  NAPHTHALENE IN POULTRY LICE PRODUCTS:
    Associate referee: Roswell Jinkins, Food and Drug Adm., Chicago, Ill.

SOILS AND LIMING MATERIALS:
  General referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

  HYDROGEN-ION CONCENTRATION:
    a. SOILS OF ARID AND SEMI-ARID REGIONS:
      Associate referee: W. T. McGeorge, Agricultural Experiment Station, Tucson, Ariz.

    b. SOILS OF HUMID REGIONS:
      Associate referee: E. R. Purvis, Virginia Truck Experiment Station, Norfolk, Va.

  LESS COMMON METALS IN SOILS:
    Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

  SELENIUM IN SOILS:
    Associate referee: K. T. Williams, Bureau of Chemistry and Soils, Washington, D. C.

  LIMING MATERIALS:
    Associate referee: W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

FEEDING STUFFS:
  General referee: L. S. Walker, Agricultural Experiment Station, Burlington, Vt.

SAMPLING
Associate referee: L. M. Jeffers, Dept. of Agriculture, Sacramento, Calif.

ASH:
Associate referee: J. L. St. John, Agricultural Experiment Station, Pullman, Wash.

MINERAL MIXED FEEDS (calcium and iodine):
Associate referee: H. E. Perkins, Manhattan, Kans.

LACTOSE IN MIXED FEEDS:
Associate referee: D. A. Magraw, American Dry Milk, Inst., Chicago, Ill.

MOISTURE:
Committee: H. A. Halvorson, P. B. Curtis, and P. A. Clifford.

HYDROCYANIC ACID IN GLUCOSIDE-BEARING MATERIALS:
Associate referee: R. A. Greene, University of Arizona, Tucson, Ariz.

BIOLOGICAL METHODS FOR DETERMINATION OF VITAMIN D CARRIERS:
Associate referee: C. D. Tolle, Food and Drug Adm., Washington, D. C.

BIOLOGICAL METHODS FOR VITAMIN B COMPLEXES:
Associate referee: O. L. Kline, Food and Drug Administration, Washington, D. C.

TECHNIC AND DETAILS OF BIOLOGICAL METHODS, VITAMIN D CARRIERS:
Associate referee: Rebecca Hubbell, Agricultural Experiment Station, New Haven, Conn.

MANGANESE:
Associate referee: J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

CAROTENE:
Associate referee: V. E. Munsey, Food and Drug Adm., Washington, D. C.

FAT IN FISH MEAL:
Associate referee: R. W. Harrison, Bureau of Fisheries, Seattle, Wash.

ADULTERATION OF CONDENSED MILK PRODUCTS AND COD-LIVER OIL:
Associate referee: P. B. Curtis, Agricultural Experiment Station, Lafayette, Ind.

FERTILIZERS:
General referee: G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

PHOSPHORIC ACID:
Associate referee: W. H. Ross, Bureau of Chemistry and Soils, Washington, D. C.

NITROGEN:
Associate referee: A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

MAGNESIUM AND MANGANESE:
>   *Associate referee:* J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

POTASH:
>   *Associate referee:* O. W. Ford, Agricultural Experiment Station, Lafayette, Ind.

ACID AND BASE-FORMING QUALITY:
>   *Associate referee:* L. E. Horat, Agricultural Experiment Station, Lafayette, Ind.

CALCIUM, SULFUR, COPPER, ZINC:
>   *Associate referee:* Gordon Hart, Department of Agriculture, Tallahassee, Fla.

PLANTS:
>   *General referee:* E. J. Miller, Agricultural Experiment Station, E. Lansing, Mich.

LESS COMMON METALS:
>   *Associate referee:* J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

CARBOHYDRATES:
>   *Associate referee:* J. T. Sullivan, U. S. Regional Pasture Research Lab., State College, Pa.

INULIN:
>   *Associate referee:* T. G. Phillips, University of New Hampshire, Durham, N. H.

FORMS OF NITROGEN:
>   *Associate referee:* H. B. Vickery, Agricultural Experiment Station, New Haven, Conn.

HYDROCYANIC ACID:
>   *Associate referee:* R. A. Greene, University of Arizona, Tucson, Ariz.

SODIUM AND POTASSIUM:
>   *Associate referee:* R. T. Milner, Regional Soybean Industrial Products Lab., Urbana, Ill.

LIGNIN:
>   *General referee:* M. Phillips, Bureau of Chemistry and Soils, Washington, D. C.

ENZYMES:
>   *General referee:* A. K. Balls, Bureau of Chemistry and Soils, Washington, D. C.

PAPAIN:
>   *Associate referee:* R. R. Thompson, Hawaiian Experiment Station, Honolulu, Hawaii.

PAINTS, PAINT MATERIALS AND VARNISHES:
>   *General referee:* C. S. Ladd, Food Commissioner and Chemist, Bismarck, N. D.

ACCELERATING TESTING OF PAINTS:
>   *Associate referee:* L. L. Carrick, Agricultural Experiment Station, Fargo
>   N. D.

VARNISHES:
>   *Associate referee:* F. Roberts, Paint and Varnish Lab., Bismarck, N. D.

VITAMINS:
>   *General referee:* E. M. Nelson, Food and Drug Adm., Washington, D. C.

   VITAMIN A:
>   *Associate referee:* J. B. Wilkie, Food and Drug Adm., Washington, D. C.

   VITAMIN D:
>   *Associate referee:* W. C. Russell, Agricultural Experiment Station, New
>   Brunswick, N. J.

   VITAMIN K:
>   *Associate referee:* H. J. Almquist, University of California, Berkeley, Calif.

   RIBOFLAVIN:
>   *Associate referee:* A. R. Kemmerer, Agricultural Experiment Station, Col-
>   lege Station, Texas.

LEATHERS AND TANNING MATERIALS:
>   *General referee:* I. D. Clarke, Bureau of Chemistry and Soils, Washington,
>   D. C.

DISINFECTANTS:
>   *General referee:* C. N. Brewer, Food and Drug Adm., Washington, D. C.

NAVAL STORES:
>   *General referee:* V. E. Grotlisch, Food and Drug Adm., Washington, D. C.

RADIOACTIVITY:
>   *General referee:* C. H. Badger, Food and Drug Adm., Washington, D. C.

   QUANTUM COUNTER:
>   *Associate referee:* A. E. Mix, Food and Drug Adm., Washington, D. C.

   GAMMA RAY SCOPE:
>   *Associate referee:* C. H. Badger.

COSMETICS:
>   *General referee:* E. W. Campbell, Bureau of Health, Augusta, Me.

DRUGS:
>   *General referee:* L. E. Warren, Food and Drug Adm., Washington, D. C.

   ACETYLSALICYLIC ACID, ACETOPHENETIDIN, AND SALOL:
>   *Associate referee:* D. C. Grove, Food and Drug Adm., Washington, D. C.

   AMINOPYRINE AND PHENOBARBITAL IN MIXTURES:
>   *Associate referee:* E. C. Payne, Food and Drug Adm., Chicago, Ill.

   BIOLOGICAL TESTING:
>   *Associate referee:* J. C. Krantz, Jr., University of Maryland, College Park,
>   Md.

DAPHNIA METHODS:
- *Associate referee:* A. Viehoever, Philadelphia, Pa.

ELIXIR OF TERPIN HYDRATE AND CODEINE:
Associate referee: Jonas Carol, Food and Drug Adm., Cincinnati, O.

ERGOT ALKALOIDS:
Associate referee: Lloyd C. Miller, Food and Drug Adm., Washington, D. C.

GUAIACOL IN MIXTURES:
Associate referee: K. L. Milstead, Food and Drug Adm., Chicago, Ill.

PHYSOSTIGMINE SALICYLATE:
Associate referee: G. M. Johnson, Food and Drug Adm., St. Louis, Mo.

SEPARATION OF ACETANILID AND SALOL:
Associate referee: O. C. Kenworthy, Food and Drug Adm., New York City.

ARECOLINE HYDROBROMIDE:
Associate referee: H. R. Bond, Food and Drug Adm., Chicago, Ill.

IODINE OINTMENT:
Associate referee: W. F. Reindollar, State Dept. of Health, Baltimore, Md.

MICROCHEMICAL TESTS FOR ALKALOIDS:
Associate referee: C. K. Glycart, Food and Drug Adm., Chicago, Ill.

MICROCHEMICAL TESTS FOR SYNTHETICS:
Associate referee: I. S. Shupe, Food and Drug Adm., Kansas City.

BENZEDRINE:
Associate referee: J. H. Cannon, Food and Drug Adm., St. Louis, Mo.

OINTMENT OF YELLOW MERCURIC OXIDE:
Associate referee: H. O. Moraw, Food and Drug Adm , Chicago, Ill.

THEOPHYLLINE SODIUM SALICYLATE:
Associate referee: M. L. Harris, Food and Drug Adm., Houston, Texas.

MANDELIC ACID MIXTURES:
Associate referee: H. G. Underwood, Food and Drug Adm., Cincinnati, Ohio.

RHUBARB AND RHAPONTICUM:
Associate referee: E. H. Wirth, University of Illinois, Chicago, Ill.

PLASMOCHINE:
Associate referee: F. C. Sinton, Food and Drug Adm., New York City.

HYDROXYQUINOLINE SULFATE:
Associate referee: W. H. Hartung, State Dept. of Health, Baltimore, Md.

PEPSIN:
Associate referee: E. M. Hoshall, Food and Drug Adm., Baltimore, Md.

IPECAC AND OPIUM POWDER (Dover's powder):
Associate referee: W. F. Kunke, Food and Drug Adm., Chicago, Ill.

NICOTINIC ACID:
> *Associate referee:* P. S. Jorgensen, Food and Drug Adm., San Francisco, Calif.

EPHEDRINE IN JELLIES:
> *Associate referee:* E. H. Grant, Food and Drug Adm., Boston, Mass.

PURIFICATION OF CAFFEINE IN PLANT EXTRACTIVES:
> *Associate referee:* John R. Matchett, Bureau of Narcotics, Washington, D. C.

DAIRY PRODUCTS:
> *General referee:* G. G. Frary, Dairy and Food Dept., Vermillion, S. D.

BUTTER:
> *Associate referee:* J. A. Mathews, Food and Drug Adm., Washington, D. C.

CHEESE (isolation of fat):
> *Associate referee:* I. D. Garard, Rutgers University, New Brunswick, N. J.

MALTED MILK (fat):
> *Associate referee:* E. W. Coulter, Food and Drug Adm., Chicago, Ill.

MALTED MILK (casein):
> *Associate referee:* I. Schurman, Food and Drug Adm., Chicago, Ill.

DRIED MILK (lactic acid):
> *Associate referee:* F. Hillig, Food and Drug Adm., Washington, D. C.

FROZEN DESSERTS:
> *Associate referee:* M. J. Mack, Massachusetts State College, Amherst, Mass.

LACTOSE IN MILK:
> *Associate referee:* E. R. Garrison, University of Missouri, Columbia, Mo.

MOLD IN BUTTER:
> *Associate referee:* J. D. Wildman, Food and Drug Adm., Washington, D. C.

TESTS FOR PASTEURIZATION OF MILK AND CREAM:
> *Associate referee:* F. W. Gilcreas, Department of Health, Albany, N. Y.

TESTS FOR PASTEURIZATION OF BUTTER:
> *Associate referee:* E. H. Parfitt, Purdue Unversity, Lafayette, Ind.

DIFFERENCE BETWEEN DAIRY PRODUCTS MADE FROM COW'S MILK AND THOSE MADE FROM THE MILK OF OTHER ANIMALS:
> *Associate referee:* I. D. Garard.

DECOMPOSITION IN DAIRY PRODUCTS:
> *Associate referee:* C. S. Myers, Food and Drug Adm., Washington, D. C.

NEUTRALIZERS IN DAIRY PRODUCTS:
> *Associate referee:* F. Hillig.

OILS, FATS AND WAXES:
> *General referee:* G. S. Jamieson, Bureau of Chemistry and Soils, Washington, D. C.

REFRACTOMETRIC DETERMINATION OF OIL IN SEEDS:
Associate referee: Lawrence Zeleny, Bureau of Agricultural Economics, Washington, D. C.

THIOCYANOGEN NUMBER:
Associate referee: G. S. Jamieson.

POLENSKI NUMBER:
Associate referee: R. S. McKinney, Bureau of Chemistry and Soils, Washington, D. C.

EGGS AND EGG PRODUCTS:
General referee: E. O. Haenni, Food and Drug Adm., Washington, D. C.

UNSAPONIFIABLE CONSTITUENTS AND FAT:
Associate referee: E. O. Haenni.

DETECTION OF DECOMPOSITION (glycerol and sugars):
Associate referee: L. C. Mitchell, Food and Drug Adm., St. Louis, Mo.

METALS IN FOODS:
General referee: H. J. Wichmann, Food and Drug Adm., Washington, D. C.

ARSENIC AND ANTIMONY:
Associate referee: C. C. Cassil, Bureau of Entomology and Plant Quarantine, Washington, D. C.

COPPER:
Associate referee: D. L. Drabkin, University of Pennsylvania, Philadelphia, Pa.

ZINC:
Associate referee: W. S. Ritchie, Agricultural Experiment Station, Amherst, Mass.

FLUORINE:
Associate referee: P. A. Clifford, Food and Drug Adm., Washington, D. C.

LEAD:
Associate referee: P. A. Clifford.

MERCURY:
Associate referee: W. O. Winkler, Food and Drug Adm., Washington, D. C.

SELENIUM:
Associate referee: A. K. Kline, Food and Drug Adm., San Francisco, Calif.

FUMIGATION RESIDUES IN FOODS:
Associate referee: W. O. Winkler.

COLORING MATTERS IN FOODS:
General referee: C. F. Jablonski, Food and Drug Adm., New York City.

FRUITS AND FRUIT PRODUCTS:
General referee: B. G. Hartmann, Food and Drug Adm., Washington, D. C.

ELECTROMETRIC TITRATION OF ACIDS:
Associate referee: R. U. Bonnar, Food and Drug Adm., Washington, D. C.

MALIC, ISOCITRIC, AND LACTIC ACIDS:
  Associate referee: B. G. Hartmann.

P_2O_5 IN JAMS, JELLIES, AND OTHER FRUIT PRODUCTS:
  Associate referee: H. W. Gerritz, Food and Drug Adm., San Francisco, Calif.

POLARISCOPIC METHODS FOR, AND ASH IN, JAMS, JELLIES, AND PRESERVES:
  Associate referee: R. A. Osborn, Food and Drug Adm., Washington, D. C.

CANNED FOODS:
  General referee: V. B. Bonney, Food and Drug Adm., Washington, D. C.

TOMATO PRODUCTS:
  Associate referee: L. M. Beacham, Jr., Food and Drug Adm. Washington, D. C.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:
  General referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

ORGANIC SOLVENTS IN FLAVORS:
  Associate referee: R. D. Stanley, Food and Drug Adm., Chicago, Ill.

MEATS AND MEAT PRODUCTS:
  General referee: R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

CACAO PRODUCTS:
  General referee: W. O. Winkler, Food and Drug Adm., Washington, D. C.

GUMS IN FOODS:
  General referee: F. Leslie Hart, Food and Drug Adm., Los Angeles, Calif.

STARCHY FOODS:
  Associate referee: D. D. Ballard, Food and Drug Adm., San Francisco, Calif.

SPICES AND CONDIMENTS:
  General referee: S. Alfend, Food and Drug Adm., St. Louis, Mo.

VOLATILE CONSTITUENTS:
  Associate referee: J. F. Clevenger, Food and Drug Adm., New York City.

VINEGAR:
  Associate referee: A. M. Henry, Food and Drug Adm., Atlanta, Ga.

SALAD DRESSINGS:
  Associate referee: L. T. Ryan, N. Dakota Regulatory Laboratory, Bismarck, N. D.

MICROBIOLOGICAL METHODS:
  General referee: A. C. Hunter, Food and Drug Adm., Washington, D. C.

CANNED FISH PRODUCTS:
  Associate referee: O. W. Lang, Hooper Foundation Medical Research, University of California, San Francisco, Calif.

CANNED MEATS:
  Associate referee: L. B. Jensen, Swift & Co., Chicago, Ill.

CANNED VEGETABLES:
> Associate referee: E. J. Cameron, National Canners Assn., Washington, D. C.

CANNED TOMATO PRODUCTS:
> Associate referee: B. A. Linden, Food and Drug Adm., Washington, D. C.

SUGAR:
> Associate referee: E. J. Cameron.

EGGS AND EGG PRODUCTS:
> Associate referee: Roy Schneiter, Food and Drug Adm., Washington, D. C.

FISH AND OTHER MARINE PRODUCTS:
General referee: H. D. Grigsby, Food and Drug Adm., Philadelphia, Pa.

SOLIDS AND FATS:
> Associate referee: R. W. Stewart, Food and Drug Adm., Philadelphia, Pa.

SUGARS AND SUGAR PRODUCTS:
General referee: R. F. Jackson, National Bureau of Standards, Washington, D. C.

ACETYL-METHYL CARBINOL AND DIACETYL IN FOOD PRODUCTS:
> Associate referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

SUGARS IN MOLASSES:
> Associate referee: F. W. Zerban, Sugar Trade Lab., New York City.

SUCROSE AND ASH IN MOLASSES:
> Associate referee: R. A. Osborn, Food and Drug Adm., Washington, D. C.

HONEY:
> Associate referee: R. E. Lothrop, Bureau of Chemistry and Soils, Washington, D. C.

MAPLE PRODUCTS:
> Associate referee: J. J. Perlman, Dept. of Agriculture and Markets, Albany, N. Y.

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS:
> Associate referee: C. F. Snyder, National Bureau of Standards, Washington, D. C.

POLARISCOPIC METHODS (GENERAL):
> Associate referee: R. M. Kingsbury, Bureau of Chemistry and Soils, Washington, D. C.

CHEMICAL METHODS FOR REDUCING SUGARS:
> Associate referee: R. F. Jackson.

REFRACTIVE INDICES OF SUGAR SOLUTIONS:
> Associate referee: R. T. Balch, Bureau of Chemistry and Soils, Washington, D. C.

WATERS, BRINE, AND SALTS:
General referee: A. E. Mix, Food and Drug Adm., Washington, D. C.

EFFERVESCENT SALTS:
Associate referee: A. E. Mix.

CEREAL FOODS:
General referee: V. E. Munsey, Food and Drug Adm., Washington, D. C.

ASH IN FLOUR, MACARONI PRODUCTS, AND BAKED PRODUCTS:
Associate referee: L. H. Bailey, Bureau of Chemistry and Soils, Washington, D. C.

H-ION CONCENTRATION OF FLOUR:
Associate referee: George Garnatz, The Kroger Food Foundation, Cincinnati, Ohio.

ACIDITY OF FLOUR:
Associate referee: Lawrence Zeleny, Bureau of Agricultural Economics, Washington, D. C.

STARCH IN FLOUR:
Associate referee: C. Y. Hopkins, National Research Council, Ottawa, Canada.

SUGAR IN FLOUR:
Associate referee: R. M. Sanstedt, Agricultural Experiment Station, Lincoln, Nebr.

BAKING TEST FOR SOFT WHEAT FLOUR:
Associate referee: E. G. Bayfield, Agricultural Experiment Station, Wooster, Ohio.

FLOUR-BLEACHING CHEMICALS:
Associate referee: Dorothy Scott, Food and Drug Adm., New York City.

CO_2 IN SELF-RISING FLOUR:
Associate referee: Rufus A. Barackman, Victor Chem. Works, Chicago Heights, Ill.

MILK SOLIDS IN MILK BREAD:
Associate referee: V. E. Munsey.

COLD WATER EXTRACT FLOUR:
Associate referee: H. C. Fellows, Bureau of Agricultural Economics, Washington, D. C.

ERGOT IN FLOUR:
Associate referee: Lloyd C. Miller, Food and Drug Adm., Washington, D. C.

PROTEOLYTIC ENZYMES:
Associate referee: Quick Landis, Fleischmann Labs., New York City.

COLOR IN FLOUR:
Associate referee: H. K. Parker, Novadel-Agene Corporation, Newark, N. J.

SOYA FLOUR IN FOODS:
>*Associate referee:* J. W. Hayward, Archer-Daniels-Midland Co., Milwaukee, Wisc.

WHOLE WHEAT FLOUR:
>*Associate referee:* C. S. Ladd, N. Dakota Regulatory Dept., Bismarck, N. D.

PHOSPHATED FLOUR:
>*Associated referee:* J. R. Davies, General Foods Corp., Chicago, Ill.

STEROLS:
>*Associate referee:* E. O. Haenni, Food and Drug Adm., Washington, D. C.

CORN PRODUCTS:
>*Associate referee:* Lyle Brown, A. E. Staley Mfg. Co., Decatur, Ill.

OAT PRODUCTS:
>*Associate referee:* H. P. Howells, Quaker Oats Co., Cedar Rapids, Iowa.

RYE AND BUCKWHEAT:
>*Associate referee:* E. G. Harrel, Pillsbury Flour Mills Co., Minneapolis, Minn.

BARLEY AND RICE:
>*Associate referee:* Allen D. Dickson, Bureau of Plant Industry, Madison, Wisc.

BAKED PRODUCTS OTHER THAN BREAD:
>*Associate referee:* S. Voris, Loose-Wiles Biscuit Co., Long Island City, N. Y.

BAKING POWDERS—TARTRATES:
General referee: B. G. Hartmann, Food and Drug Adm., Washington, D. C.

MICROCHEMICAL METHODS:
Associate referee: E. P. Clark, Bureau of Entomology and Plant Quarantine, Washington, D. C.

ALCOHOLIC BEVERAGES:
General referee: J. W. Sale, Food and Drug Adm., Washington, D. C.

DIASTATIC ACTIVITY OF MALT:
>*Associate referee:* Christian Rask, Albert Schwill Co., Chicago, Ill.

PROTEOLYTIC ACTIVITY OF MALT:
>*Associate referee:* Stephen Laufer, Schwartz Laboratories, Inc., New York City.

HEAVY METALS IN BEER·
>*Associate referee:* W. H. Harrison, Continental Can Co., Chicago, Ill.

CARBON DIOXIDE IN BEER:
>*Associate referee:* P. P. Gray, Wallerstein Laboratories, New York City.

MALT EXTRACT IN MALT:
>*Associate referee:* E. A. Siebel, 8 S. Dearborn St., Chicago, Ill.

MALT ADJUNCTS:
>*Associate referee:* F. P. Siebel, Siebel Institute, Chicago, Ill.

BEER:
> *Associate referee:* H. W. Rohde, Schlitz Brewing Co., Milwaukee, Wisc.

TOTAL SULFUR IN WINE:
> *Associate referee:* B. G. Hartmann, Food and Drug Adm., Washington, D. C.

VOLATILE ACIDS IN WINE:
> *Associate referee:* M. A. Joslyn, Agricultural Experiment Station, Berkeley, Calif.

SULFUR DIOXIDE IN BEER AND WINE:
> *Associate referee:* L. V. Taylor, American Can Co., Maywood, Ill.

VOLATILE ACIDS IN DISTILLED SPIRITS:
> *Associate referee:* G. F. Beyer, Bureau of Internal Revenue, Washington, D. C.

ALDEHYDES IN WHISKEY AND OTHER POTABLE SPIRITS:
> *Associate referee:* Peter Valaer, Bureau of Internal Revenue, Washington, D. C.

DETECTION OF ADULTERATION OF DISTILLED SPIRITS:
> *Associate referee:* S. T. Schicktanz, Bureau of Internal Revenue, Washington, D. C.

WOOD ALCOHOL IN BRANDY:
> *Associate referee:* G. F. Beyer.

CORDIALS AND LIQUEURS:
> *Associate referee:* J. B. Wilson, Food and Drug Adm., Washington, D. C

FOOD PRESERVATIVES—SACCHARIN:
> *General referee:* W. F. Reindollar, Bureau of Chemistry, Baltimore, Md.

BENZOATE OF SODA:
> *Associate referee:* A. E. Mix, Food and Drug Adm., Washington, D. C.

NUTS AND NUT PRODUCTS:
> *General referee:* S. C. Rowe, Food and Drug Adm., Washington, D. C.

COFFEE AND TEA:
> *General referee:* H. J. Fisher, Agricultural Experiment Station, New Haven, Conn.

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WILEY MEMORIAL LECTURE. No. IX
THE HISTORY AND DEVELOPMENT OF FOOD INSPECTION IN THE UNITED STATES*

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When it was suggested that the subject of this address should relate to the early history of food inspection in the United States, culminating in the passage of the Food and Drugs Act of June 30, 1906, and to the organization for the enforcement of this law, particularly the part played by Dr. Wiley in bringing this about, it seemed an especially appropriate title at this time, because a new Food and Drugs Act has just been passed after a long struggle, and again the work ahead is the development of new regulations and the organization of new groups to carry it into effect. At this time we could well look back over the experience of the formative period of food inspection, and review some of the difficulties, troubles, and mistakes that were made, with the idea of benefiting from these in connection with the present problem.

It is a privilege and honor to be asked by this Association to present this Wiley Memorial Lecture, and I have enjoyed going back over its proceedings and also the work of the Bureau of Chemistry, in order to obtain information necessary to bring out the facts leading to the passage of the original Food and Drugs Act and its organization.

EARLY FOOD INSPECTION AND LEGISLATION

It is not my intention to go deeply into the history of food inspection in the United States, but I shall review briefly the work of this Association, of the Bureau of Chemistry, and of Dr. Wiley and his associates, and the part that they played in bringing about the passage of the Food and Drugs Act. It is desirable, however, to call attention to the different methods used in early food legislation.

For a number of years, under its taxing power, the Bureau of Internal Revenue was the principal enforcer of food legislation. The first important legislation was the Act of August 2, 1886, which taxed oleomargarine, renovated butter, and adulterated butter, and which gave the Bureau of Internal Revenue complete authority over these products. With the power that accompanies the taxing authority, revenue agents were allowed to enter any plant and follow the product, whether it went into interstate commerce or only into intrastate commerce. Anyone who has had experience with the enforcement of National food legislation based solely upon interstate commerce control will realize what an effective power the Bureau of Internal Revenue had for enforcing this law. The only interest that the Bureau of Internal Revenue had in the matter,

however, was the collection of taxes, and in this particular legislation the purpose was not to collect taxes, but to tax out of existence certain products like colored oleomargarine, etc. Similarly, the Act of June 6, 1896, taxing filled cheese, and the Act of June 13, 1898, taxing mixed flour, were primarily for the purpose of preventing adulteration of these products by placing a tax upon the adulterated product that would practically take it off the market.

In addition to these special acts, the Bureau of Internal Revenue was concerned with the adulteration of whiskey, and was able to control this product under its taxing power. While I was Chief Chemist of this Bureau I was greatly impressed with this power of control by the taxing method, and I often thought that if Dr. Wiley had just happened to be attached to the Bureau of Internal Revenue or the Treasury Department we might have seen an entirely different development of Food Inspection in the United States.

Another early method of Food Inspection is brought out in the Act on Inspection of Tea, March 2, 1897. The act was primarily enforced by the Customs. In other words, all tea being imported is regulated as to its purity at the Customs, and is detained until examined and passed. It was this power of the Customs inspectors that was used in connection with the imported food products under the Federal Food and Drugs Act.

Another most interesting forerunner of the Food and Drugs Act was the Act of August 30, 1890, which was passed primarily to control meat for export, but which also included sections covering the importation of adulterated food products and liquors. This law was peculiar in that it made the person importing the product liable and subject to fine. It also had a great weakness so far as enforcement was concerned in that it included the following provision:

Any person who shall *knowingly* import into the United States any such adulterated food or drug or drink, knowing or having reasons to believe the same to be adulterated, being the owner or the agent of the owner, or the consignor or consignee of the owner, or in privity with them, assisting in such unlawful act shall be deemed guilty of misdemeanor and liable to prosecution therefor in the district court of the United States for the district into which such property is imported.

The difficulty lay in having to prove guilty knowledge. Although this Act of 1890 and the later Act of 1891 were primarily the beginning of meat inspection, dairy products intended for export were also covered in its provisions. This sort of review of these preliminary Acts is very significant in a study of the development of the Food and Drugs Act.

Many interesting details regarding the legislation at this time will be found in Bulletin 69 of the Bureau of Chemistry, by W. D. Bigelow, which includes a brief review of food legislation. The national laws passed up to 1896–7 were largely directed at specific products, such as butter,

cheese, flour, etc. In this respect the national legislation had followed much the same course as had food legislation in the various states, which had passed specific measures covering such products as butter, vinegar, honey, etc., rather than general laws.

In this Association of Official Agricultural Chemists active interest in food adulteration began about 1896. In a review of its proceedings for 1894 we find only a few references to the subject of food adulteration, but the proceedings of 1895 show that the Association took a definite step in the study of food adulteration and an active interest in promoting food legislation. A Committee on Food Legislation was appointed by A. L. Winton, president at that time. It consisted of H. W. Wiley, Chairman; H. A. Huston, Lafayette, Ind.; J. A. Myers, Morgantown, W.Va., and A. S. Mitchell of Milwaukee, Wis.

The proceedings of 1896 have little information on this subject, but in the proceedings of 1897 there first appears among the list of referees a Referee on Food Adulteration. The Committee on Food Standards, of which Dr. Wiley was chairman, also makes its first appearance. The first report by the Referee on Food Adulteration in 1897 is quite different from later reports. Instead of dealing with methods for the detection of adulteration and the analysis of products, it combines a report on definitions and standards with a list of adulterations, and an attempt was made to give standards of composition that would show that a product was not normal. Little attention was devoted to the study of methods of analysis.

In a discussion of this report, Dr. L. L. Van Slyke made the following statement, which I think is worthy of reading at this time, showing as it does the importance attached to standards by the Association at the very outset: "If the Association adopts standards they will be made use of in the Courts of different States. If we make a mistake we shall do an injury because our work will be referred to by one side or the other, and if in the suggestion of a standard we make an error, then somebody will make use of it to our injury as well as to the injury of the trade in which that particular product may be found."

HISTORY OF FOOD STANDARDS

The study of food standards began with this first report of the referee in 1897, and it has also been interesting to me to review the various steps that have been taken in their formation, the changes in principles that have come about from time to time, and the care that is necessary to establish proper limits. As an illustration of this, I think that it is worth while to follow the standards established for cider vinegar through the various editions of the definitions that have been issued from 1903 up to the present.

Vinegar, cider vinegar, or apple vinegar.—Cider vinegar is the product made by the alcoholic and subsequent acetous fermentations of the juice of apples, is laevo-rotatory, and contains not less than four (4) grams of acetic acid, not less than one and six-tenths (1.6) grams of apple solids, and not less than twenty-five hundredths (.025) gram of apple ash in one hundred (100) cubic centimeters. The water-soluble ash from one hundred (100) cubic centimeters of the vinegar requires not less than thirty (30) cubic centimeters of deci-normal acid to neutralize the acidity, and contains not less than ten (10) milligrams of phosphoric acid (P_2O_5).

This standard, which was in force for many years, actually compelled the manufacturer of high-grade cider vinegar to adulterate his product in order to meet the requirements. In a later edition there was injected into this definition of cider vinegar the provision that not more than 50 per cent of these apple solids should be reducing sugars, which simply added to the confusion because the amount of reducing sugars depended entirely upon whether or not the proper fermentation of the cider had originally taken place. When a careful study was made of this subject and complete fermentation had been effected, very small amounts of sugar were left in the solids and a more satisfactory and stable product was produced, but it frequently contained less than 1.6 per cent of solids. In the 4th revision of these definitions we find that the standard for cider vinegar has been revised to a simple status, and defined as a product "made by the alcoholic and subsequent acetous fermentation of the juice of apples and containing not less than four (4) grams of acetic acid." Thus has evolved through many years of change a definition or standard that is undoubtedly correct.

A comparison of the first report on food standards with subsequent reports is also interesting. In the beginning an effort was made to include data to simplify the work of the chemists or the analysts in determining adulteration. In most cases, however, this inclusion of additional data was of no material benefit, and as a rule served merely as a guide to the manufacturer who wished to adulterate his product and conceal the adulteration by meeting these standards. As our experience has increased a tendency towards simplification and exact definition has been apparent. The minimum standards are included only in case the distinctive ingredient of the product can be identified and the proper limit on it prescribed such as the standard of 5 per cent of oil of lemon in lemon extract.

Since the new Federal Food, Drug, and Cosmetic Act gives authority to the Secretary of Agriculture to promulgate regulations fixing and establishing definitions and standards and since after promulgation they have the authority of law, the new Committee on Standards should exercise the greatest care in their preparation. I think it will be found that most of the mistakes that occurred in the standards in the past were in connection with the data either analytical or inspectional included in the standards to support the definition.

I note that Section 401 of the new Act covering the Definition and Standards for Food, states that—

Whenever in the judgment of the Secretary such action will promote honesty and fair dealing in the interest of consumers, he shall promulgate regulations fixing and establishing for any food, under its common or usual name so far as practicable, a reasonable definition and standard of identity, a reasonable standard of quality and/or reasonable standards of fill of container.

As you will note, the wording of the law states that it is entirely in the interests of *consumers*, but as a matter of fact, these standards have a tremendous effect upon *industry* and fair trade and industry is just as anxious that these standards shall be correct as either the Government or the consumer, and is ready to cooperate in making them correct.

From 1896 to the present time the proceedings of this Association show a gradual but rapid development of the functions of the Referee on Food Adulteration, and today page after page of our program is allotted to reports of referees and associate referees on various phases of food and drug adulteration.

CONTACT WITH DR. WILEY

My first contact with Dr. Wiley and his associates was in January, 1900, when I came to the Bureau, then known as the Division of Chemistry, and began work on the examination of canned meats, which study was an outgrowth of the trouble with this product encountered during the Spanish-American war. This work was later published as a part of Bulletin 13, Foods and Food Adulterants. It would be advantageous for anyone to review some of the bulletins published at that time and especially this one on meats, because it contains much valuable information on the subject of fresh and canned meats, and also a discussion of the then known methods for canning meats.

The years from 1900 to 1906 constituted a period of great activity on the part of the Bureau of Chemistry and of the Association in studies of food adulteration and methods for its detection. The work was primarily directed towards getting the information necessary for Congress to justify the passage of the National Food and Drugs Act. During this period, accordingly, the proceedings of this Association show marked expansion. New associate referees on different products were constantly being added to the list and the Association's interest in food and drug adulteration was becoming the dominant factor. It was during this period that the first important food inspection laboratories were established, and they cooperated with the Customs service in examination of imported food products. The first of these laboratories was located at the Port of New York, under R. E. Doolittle.

Under the regulation of imported foods, samples were taken and the products were detained at the port of entry until examinations could be made, and then they were either released, relabeled, or refused entry. This examination of imported food products was valuable training for the group of young and inexperienced analysts who had accessible little

in the way of methods and standards upon which to base their judgment. Much time was given to the inspection of such products as olive oil, wines, and the various types of canned goods. I shall never forget the criticism that Dr. Wiley made of E. M. Chace and myself in regard to our method of opening champagne bottles. He came into the laboratory and showed me with great skill how the cork could be removed from the bottle of champagne without disturbing its content of gas.

Another personal recollection of this time is that of helping Dr. Wiley prepare to appear before the Interstate Commerce Committee of the House of Representatives in support of the then pending Food and Drugs Act. I was reminded of this when reading from the address on "Wiley the Teacher," by W. W. Skinner, in which he mentions how he and J. K Haywood had worked feverishly Saturday, Sunday, and Monday preparing literature for Dr. Wiley and how they had collected the enormous amount of data and references to which he listened, but regarding which he took no notes. When the time came for presenting the facts, however, Dr. Wiley was able to cite the literature and other references. Likewise, three young fellows connected with the food laboratory (L. S. Munson, E. M. Chace, and myself) worked for weeks and prepared so many exhibits of adulterated foods and of books of references that at the time of the hearing they had to hire a horse and truck to carry all this material to the room of the Committee of Interstate Commerce and arrange it for Dr. Wiley's use. For two days these assistants stood around waiting to be asked to secure this and to secure that, but as a matter of fact not one single exhibit or book was used by Dr. Wiley. Apparently he had all of this material in the back of his mind, and for two days he presented the case for the Food and Drugs Act to this Committee. I know that my feeling at the beginning of this hearing was that the Committee was distinctly antagonistic to him. At the end, however, there was no doubt that the Committee had entirely changed its attitude. We had listened to a great oration on the subject of food adulteration and the need of food legislation.

MANUFACTURE OF EDIBLE GELATINE

I shall not discuss at this time problems of organization of the work, but I should like to mention some of the constructive things that were done for the benefit of food industry during this period, and one particularly with which I was intimately connected. After the passage of the Food and Drugs Act certain rules and regulations regarding the use of preservatives presented to various manufacturers problems that were exceedingly difficult to handle. For example, in the manufacture of edible gelatine the use of sulfurous acid had been universal. The raw material was treated with sulfur dioxide and sufficient sulfurous acid was present in the product during all the stages of manufacture to keep down bacterial

growth. When manufacturers of gelatine were faced with the proposition of making an edible gelatine without the use of preservatives, they did not succeed immediately in overcoming the difficulties encountered. As a result many lots of gelatine were highly contaminated with bacteria. One of the constructive methods used by Dr. Wiley and the Bureau in such a case was to send some of their scientific men out to study the problem of the industry and to determine how such a product could be manufactured in a satisfactory way without the use of preservatives. I was one of those selected to visit the various gelatine factories in the United States and to study their problems.

In making this survey of gelatine manufacturers, we went to each plant and studied the raw material and each step in the handling. We took samples at each and every stage of the operation and subjected them to bacteriological examination. In many cases we were able to find the principal point of infection quickly and to offer suggestions as to how to correct this difficulty. This particular industry had been run by rule of thumb, based on the experience of men trained in it but who had had no scientific background, and I think that this was the first time the science of bacteriology had been injected into the gelatine industry. The air filtration introduced into the drying tunnels and other improvements made in the equipment resulted in the manufacture of a much better quality of edible gelatine. The importance of building equipment that can be cleaned effectively and cheaply impressed the early inspectors, and as a result of this type of investigation many improvements in sanitation have been brought about in every type of food industry. The general principles applied at that time have been found to be universal in their application. It was also impressed upon all of us who made this investigation that valves and joints in pipe lines and pumps can be very dangerous, and cause spoilage or deterioration of the finished product. Tremendous strides in layouts of equipment and factories and the building of sanitary food machinery have been made in this country in the last few years. I do not believe that there is a single food industry in the United States that has not directly or indirectly benefited by this early constructive work on the part of the Bureau of Chemistry during the early period of the Food and Drugs Act.

Those were what might be called the pioneer days of food sanitation in this country, and if there is anything for which we should be especially thankful, it is for this constructive development in the enforcement of the Food and Drugs Act. Not only has this work improved the quality of foods, it has also been of tremendous monetary value to industry in reducing its losses due to spoilage. A review of the studies made at that time of the manufacture of ketchup, the handling of milk, and the drying of fruit alone is most convincing.

It is not necessary at this time to discuss the question as to whether or

not the use of preservatives was actually injurious to health. There is no question but that the regulations compelling the manufacturer to go ahead without them was of great importance to the food industry in developing new and better methods of food handling, as well as to the food consumer.

Another important development that came soon after the passage of the Food and Drugs Act was the forcing of the food industry to establish a scientific control. We find today as a result that most large food manufacturing concerns have their technical and research laboratories, which, without question, have proved their value. We also see the establishment during this period of such institutions as the National Canners Research Laboratory in charge of Dr. Bigelow, directing its attention primarily to problems of the canning industry. Their principal work has been along the lines of improving sanitation and character of the canning equipment, and control or prevention of spoilage.

Looking back, I consider that this was a glorious period in our food manufacturing history, and the spark plug in this whole work was Dr. Wiley. It was he who started these investigations of food manufacture with the idea of giving constructive help, and he was active in and directed most of this pioneer work. To me this is his most outstanding constructive work, and I consider that I was fortunate to have been connected with him during this period. The experience gained in the study of many food industries of the United States has been of the greatest value to me, personally.

CHANGES IN IDEAS ON DIET

In such a heterogeneous group of individuals as was brought together in those five or six years after the passage of the Food and Drugs Act, there necessarily developed many ideas and many differences of opinion. Sides were taken in a struggle that was evident within the Department of Agriculture to gain control of the enforcement of the Act, but as I look back over this period after 25 years I cannot help but feel that these struggles were in the end, beneficial, and that the problems were worked out in a much better way in the end than they would have been if these differences of opinion had not occurred and been ironed out. I am impressed with the changes in our ideas that have taken place, particularly regarding the little things in our diet. Our ideas on nutrition have undergone great changes in this period, and I think we may say that even today we are only on the threshold of knowledge on the effect of these minor constituents of our diet. The knowledge of vitamins, other food factors, and the mineral elements is daily expanding, and no one can be wise enough to foresee what the next few years may bring forth.

Recently I was reading the *Annual Review of Biochemistry* for 1936, in which Hart and Elvehjem review the present-day ideas of mineral

elements. They state that practically all workers are now agreed that copper is necessary as a supplement to iron for the formation of hemoglobin in red blooded animals. It also appears that many other mineral constituents are essential in our proper nutrition, for example, manganese, zinc, cobalt, nickel, aluminum, fluorine, bromine, iodine, and boron, but the question as to whether or not these elements in all forms are essential is widely disputed. There has been no question as to the poisonous qualities of arsenic, yet Coulson in his recent article states that many marine food products contain quantities of arsenic, which if in the condition of arsenic oxide would be poisonous, yet he calls attention to the fact that these fish foods have been eaten regularly by maritime people for centuries, which is, in his opinion, presumptive evidence that arsenic in the form that it is present in these food products is non-toxic.

Recent surveys of food products to determine the presence of these trace elements have been enlightening, and the knowledge gained suggests that we should be extremely careful in drawing conclusions as to whether or not as they occur in foods naturally they have the same poisonous character that they have when added as the chemical salts. Particularly, I think, is this true regarding fluorine as it occurs in many foods that we eat, especially the fish and other marine products. There is merit in the idea that where we find certain trace elements universally distributed in our food products we can assume that they have been present in the food of humans over many millions of years and that mankind has either obtained a tolerance to the material or that the element plays an essential part in the cell structure as it now exists.

Stefansson in his *Adventures in Diet* discusses the teeth of the Eskimo and the natives of Iceland and brings out the fact that these people, who live to a great extent on marine foods, have very fine teeth, particularly the Eskimo, who lives almost exclusively on fish. This indicates that the normally high fluorine content of the Eskimo's diet has no bearing upon the formation of mottled teeth and emphasizes the statement made by Dr. H. Trendley Dean (Public Health Report of April 16, 1935), "mottled enamel, in the light of present knowledge, is a waterborne disease, and the experimental approach should stimulate this condition."

All of this recent information regarding the difference in action of arsenic and fluorine under different conditions should certainly make us a little humble as to the absolute finality of our present-day opinions. As a matter of fact, when we consider how much there is to know regarding nutrition as compared with what we do know, we should hesitate before we reach definite conclusions.

It would not be fair to review this period without mention of the many men associated with this development of our food inspection system. A most remarkable group of men was brought together by Dr. Wiley when

he organized his work for the enforcement of the Food and Drugs Act of June 30, 1906. Take for example the first group of inspectors. We find among this list such names as Walter G. Campbell, Chief of the Food and Drug Administration; W. R. M. Wharton, Chief of the Eastern District; Dr. A. W. Bitting, the author of many books on foods and food examination; Arthur I. Judge, Editor, *Canning Age;* Jackson E. Earnshaw, now with the Harvey Company; Arthur Stengel, still with the Food and Drug Administration; F. L. Wollard, Chief of the Baltimore Station; George H. Adams, Chief of the Boston Station; and many others too numerous to mention, a most select and high class group of young men. Ten of this original group still remain active under the Food and Drug Administration.

At the same time there was brought into the Bureau a large number of chemists from various State food laboratories, among whom were R. E. Doolittle, State Analyst of Michigan; A. E. Leach, State Chemist of Massachusetts; A. S. Mitchell, State Analyst of Wisconsin; A. L. Winton, State Analyst of Connecticut; Arthur L. Sullivan, now State Food Commissioner of Maryland; Benjamin Hart from Kentucky, formerly Chief of the Western District, and later prominent in the canning industry on the Pacific Coast. I could go down the list naming a number of men still active in Food and Drug Administration work, or in food and drug work of one kind or another, who were of the first group.

In conclusion, I wish to state that it has been a great pleasure to me to prepare this paper, that my association with this great group of men during this development period was of the greatest value to me personally, and that my connection with the constructive work dealing with the problems of industry in improving methods of manufacture has been exceedingly helpful to me during my whole business career. I feel sure that those having to do with the preparation of regulations under the Food and Drugs Act of 1938 and the preparation of Food Standards under that Law, can obtain, as I have, much valuable information by reviewing the proceedings of this Association and the development and progress of the period after the passage of the Food and Drugs Act of June 30, 1906.

PRESIDENT'S ADDRESS*

CHEMISTRY AND THE UTILIZATION OF AGRICULTURAL PRODUCTS

By H. R. KRAYBILL (Department of Agricultural Chemistry, Purdue University Agricultural Experiment Station, Lafayette, Indiana)

It is just twenty-one years ago today that I first attended a meeting of the Association of Official Agricultural Chemists. At that meeting in his annual extemporaneous address Dr. Wiley placed much emphasis on the importance of increasing our food supply. We were then in the midst of the World War. Food was necessary not only to supply the need of this country but also to support our allies and their armies. A popular slogan was "Food Will Win the War." How rapidly circumstances have changed! Today we do not face the problem of producing adequate food supplies. Our chief agricultural trouble seems to lie in our inability to find markets adequate to consume the unrestricted production of our farmers.

The important role that agricultural chemistry played during the last century, in increasing the efficiency of food production and thus relieving man of the fear of starvation, is well known. On the other hand, in recent years rapid developments in organic chemical technology have led to increased substitution of the products of the oil well and mine for those of the farmer. Will chemistry in the future help to solve these problems of surpluses by finding new uses for farm products? Two of the objects of our Association are "To conduct, promote, and encourage research in chemistry in its relation to agriculture," and "To afford opportunity for the discussion of matters of interest to agricultural chemists." Therefore, it seemed appropriate for me to select as my subject "Chemistry and the Utilization of Agricultural Products," even though I am fully aware of the limits of my ability to discuss the subject adequately.

For many years agricultural chemists have been interested in the possibility of developing new industrial uses for the products of the farm. Some of the very first projects undertaken when the United States Department of Agriculture was organized dealt with this problem. In an article on the relation of chemistry to the progress of agriculture in 1899, Dr. Harvey W. Wiley stated, "The application of the principles of chemical technology to the elaboration of raw agricultural products has added a new value to the products of the farm, opened up new avenues of prosperity, and developed new staple crops." In a very interesting paper read before the meeting of the American Chemical Society at Milwaukee last September Dr. Herrick mentioned many important contributions of the United States Department of Agriculture in this field. Among these

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were the utilization of plant fibers from waste materials, including corn stalks, cotton stalks, sugar cane bagasse and other materials; the use of crop plants for paper making; the manufacture of industrial alcohol from various farm crops and waste materials; the production of cellulose from various plants; of furfural, xylose, and adhesives from pentosan containing materials; the utilization of naval stores, of starch from sweet potatoes, of citric acid from lemons, and of pectin from apple waste.

During recent years a more widespread interest has developed, as is exemplified by the Farm Chemurgic movement, the establishment at the University of Illinois, by the Federal Government in cooperation with the Agricultural Experiment Stations of twelve states, of a Regional Soybean Industrial Products Laboratory, and the recent Congressional action authorizing the establishment, by the United States Department of Agriculture, of four regional research laboratories.

What are the reasons for this increased interest in the industrial utilization of agricultural products? Apparently it arises from the general belief that the depressed condition of agriculture following the World War was due to the accumulation of surpluses of agricultural products and to a general acceptance of the viewpoint that agriculture and industry represent two interdependent groups. Many industrial leaders now believe that good industrial conditions are not apt to prevail for any considerable length of time unless agriculture is reasonably prosperous.

The proportionate part of the national income that agriculture receives had been declining constantly for many years before the World War, but these changes were gradual and offered opportunity for natural readjustments. At the close of the World War rapid changes occurred in the market demand for our agricultural products. It was only then that the surpluses became burdensome.

Six important factors contributed to this situation. First, cheap sources of the most expensive element of fertilizer (nitrogen) were made possible by the development of synthetic methods of production. Second, improved agricultural practices resulted in increased production. Third, agriculture increased its production as a result of demands arising during the World War. Fourth, the replacement of horse and mule power by mechanical power resulted in a reduction in the consumption of grain. It is estimated that the products from twenty million acres were replaced by the products of the oil wells. Fifth, the loss of foreign markets as a result of the disturbed political and economic conditions throughout the world. Sixth, with the development of organic technology there was an increased replacement of agricultural products in industry by the products of the mine and the oil well.

It has been estimated that a century ago over four-fifths of all the products used by man were from the farm. Today probably not more than one-third of the weight of all products used by man inclusive of foods and

clothing is derived from the products of the farm. The displacement of agricultural products by those of the mine and oil well began many years ago, but with the rapid development of organic technology it has become an increasingly important factor. A few examples will serve to illustrate its importance. In 1869 the dyestuff alizarine was synthesized from coal tar derivatives and produced commercially. Nine years later alizarine was produced in quantities equivalent to that which could have been produced from about four million acres of madder. To grow sufficient indigo to produce the amount that is made synthetically today would require over seven million acres of land. Synthetic resins and solvents replace such agricultural products as bones, horns, hoofs, amber, natural resins, and naval store products. The more recent development of methods for the production of many organic solvents and chemicals from petroleum products is displacing products formerly made from agricultural materials. To produce alcohol equivalent to our annual consumption of gasoline would require approximately 300,000,000 acres of corn at present average yields or about three times the ten-year average acreage.

The fields for developing industrial uses for agricultural products may be classified into three groups as follows: First, the utilization of wastes and by-products from crops; second, the development of new industrial uses for crops or crop surpluses; and third, the introduction of new crops to yield products to take the place of those now imported and used for industrial purposes.

While many excellent products can be made from crop residues, the high cost of collection and transportation to the processing plant and their relatively low value leave only a small return for the farmer. Unless the farmer can receive a price for these products that is greater than their value in maintaining the fertility of the soil the utilization of crop residues for industrial purposes offers little promise of direct aid to the farmer. Considerable work has already been done in this field. Examples are the use of bagasse, cereal straw, corn stalks, and other similar types of materials to make wall board, box board and insulating materials, and the recent development of the production of furfural and its derivatives from oat hulls. In most cases where success has been obtained the available products have arisen as by-products from a plant processing agricultural products. The waste materials have thus been assembled at the plant in the course of the marketing of the crop.

The second field, the utilization of surplus food crops as raw materials for industry offers an almost unlimited field if it were available. These raw materials comprise the products for organic synthesis of chemical and structural materials. In this field there is direct competition with the cheap products of the mine and the oil well. The raw products of agriculture are essentially carbohydrates, proteins and fats. From a technological standpoint these materials could be used as raw materials for the

manufacture of chemicals just as coal and petroleum are used. However, two important factors must be given consideration in the choice of raw materials for industry. First, the cost of the raw materials, and second, the cost of the processes involved in their use. The cost of raw materials from coal and petroleum is much lower than it is from agricultural products. It would seem as though agricultural products could be used economically only in such cases where the cheaper processing costs offset the difference in the cost of raw materials or when processes are not known for making the products from coal or petroleum. Time does not permit a detailed discussion of what has been accomplished in this field. The notable work of the corn refining industry which is well known may be cited as an illustration of what may be accomplished through research.

The third field, the introduction of new plants to take the place of imported agricultural products used for industrial purposes, seems to offer considerable promise. The total annual value of our imports of agricultural products is about one and one-half billion dollars. The larger part of these imports consists of silk, sugar, coffee, sirup, oil seeds, vegetable oils, rubber, and paper pulp. Many of these products are used for industrial purposes. In 1937 we imported approximately two and two-thirds billion pounds of vegetable oils, of which a large part was used for industrial purposes. Time will not permit a full discussion of the possibilities in this field. As an illustration I have chosen the soybean because it seems to have excellent possibilities and because, even though on a small scale, our laboratory has been engaged in a study of the chemistry and utilization of the soybean for the last ten years. For the last two years these studies have been carried out cooperatively with the U. S. Regional Soybean Industrial Products Laboratory.

The marked increase in the production of soybeans in the middle west in recent years illustrates how a new crop may be used to replace acres devoted to the production of surplus crops. In 1937, about two million acres of soybeans were harvested for seed in Illinois, Indiana, Iowa, Ohio, and Missouri. Production in this area was increased four-fold in the last eight years. The crop this year will exceed fifty million bushels. Soybeans are used largely to replace oats and corn in the rotation. Thus they help the farmer to make readjustments for the loss of feed markets resulting from the replacement of horse and mule by gasoline motive power.

Two products are obtained by processing the soybean, oil and meal. About four and one-half times more meal than oil is produced. Both products may be used for edible as well as for industrial purposes. Although the meal or proteins extracted therefrom are used in making glues, plastics, and core bindings and in paper sizing the amount used industrially today is not more than one to two per cent. A larger percentage of the oil, or about 17 per cent, was used industrially in 1937. Although the total number of pounds of soybean oil used for industrial purposes has

increased steadily, it has not kept pace with the rapid increase in production.

The soybean is an ideal crop to replace such surplus crops as oats and corn in the corn belt states, provided adequate markets can be obtained. If its uses are confined largely to foods and feeding stuffs, however, it will merely compete with other food crops. If chemical technology will develop new industrial uses for the soybean, the acreage can continue to expand.

In comparison with most seed crops the soybean is characterized by a higher protein and fat and a lower carbohydrate content. It contains almost twice the amount of protein, about twelve times the amount of fat, and approximately one-half as much carbohydrate as other legume seeds such as the navy bean or pea; and over four times more protein and about one-third as much carbohydrate as corn. It contains practically no starch. It is chiefly the large amounts and high quality of the fat and protein in the soybean that make its industrial utilization so promising.

Practically ninety per cent of the fatty acids of the soybean are unsaturated. The chief acids are linoleic (52.0–58.8 per cent) and oleic (25.9–33.7 per cent). The fatty acids of soybean oil differ from corn and cotton seed oil chiefly in that they contain linolenic acid, less palmitic and oleic acids, and more linoleic acid, and from linseed oil by containing less linoleic and linolenic and more oleic acid. In composition soybean oil lies between the food oils of corn and cotton and the drying oil linseed. It is used in either of these fields but usually at a discount. The properties of the oils are doubtless influenced by the structure of the glycerides. If the glycerides were pure glycerides of each fatty acid it should be possible to remove the glycerides of the saturated or less unsaturated fatty acids and thus improve the drying properties of the oil. Experiments in our laboratories showed that very little increase in iodine number could be obtained by chilling and filtering the oil. It is possible to hydrolyze soybean oil into its constituent fatty acids and glycerol, to separate the more unsaturated acids and resynthesize them with glycerol into an oil, and obtain increased drying rates. However, present methods are not commercially economical. Recent studies reported by the U. S. Regional Soybean Industrial Products Laboratory indicate that with suitable dryers and resins soybean oil may be used successfully in place of the faster drying oils in paints and varnishes. About nine per cent of the soybean oil produced in 1937 was used in paints and varnishes.

In 1937 over 534 million pounds of linseed oil, 43 million pounds of perilla oil, and 174 million pounds of tung oil were imported. These oils are used almost entirely for industrial purposes. Large quantities are consumed by the paint and varnish industry. If methods can be devised to use soybean oil in place of some of these oils new markets will be obtained. To produce soybean oil in equivalent amount, would require about eight million acres or four times the quantity grown last year.

In addition to the true fats, crude soybean oils contain small amounts of other compounds that are extracted or pressed out with the oils. Among the most important of these are the phosphatides, sterols, sterol glucosides, and mucilages. The crude phosphatides, sold commercially as "lecithin," are obtained from solvent-extracted oil by treatment with steam and centrifuging. They are used in margarine, mayonnaise, salad dressings, chocolates, candies, baked foods, in gasoline, in rubber, in treating leather, in creosoting wood, and in soap and cosmetic preparations. It was formerly believed that "lecithin" could be obtained only from solvent-extracted oil. We have found that a good "non-break" varnish oil may be produced from expressed oil by emulsification with water and centrifuging, and at the same time a good commercial grade of "lecithin" may be obtained.

Although these crude phosphatides are designated commercially as "lecithin," they contain only a relatively small amount of true lecithin along with cephalin and other compounds. By adsorption methods Thornton separated the phosphorus-containing compounds into a number of fractions. On the basis of the total nitrogen, phosphorus, amino nitrogen, and choline contents of these fractions he has shown that not near all of the phosphorus-containing compounds can be accounted for as lecithin and cephalin. A method has been devised to refine soybean oil and at the same time recover various fractions of the phosphatides, foaming agents, emulsifying agents, sterol glucosides, and a considerable portion of the free sterols.

The sterols consist of several sitosterols and stigmasterol. These compounds are in great demand at the present time because they may be used as starting materials in the synthesis of certain sex hormones. Because of its chemical structure stigmasterol is best suited for this purpose. Apparently soybeans are the only commercial crop grown in this country in which stigmasterol is found in appreciable quantities. Since stigmasterol occurs in very small quantities in the soybean oil it is difficult to prepare in large quantities. None is produced commercially in this country. Our sole commercial source is from Germany. Crude soybean sterols have been imported for scientific studies. By the methods worked out in our laboratories about one pound of sterols containing from one-fifth to one-fourth of a pound of stigmasterol may be recovered from a ton of the crude soybean oil. A large part of the sterols may be recovered by direct crystallization from the concentrate without saponification of any of the fat.

Only a small part of the phosphatides and none of the sterols are recovered from the soybean oil produced in this country today. The possibilities of finding new and extensive uses for these products are very encouraging.

As mentioned earlier about four and one-half times more meal than oil is produced. If soybean acreage is to expand, markets must be provided for the meal as well as the oil. Recent studies reported by the U. S. Regional Soybean Industrial Products Laboratory indicate that soybean protein and oil meal may find new uses in the plastic field. Reports from industrial laboratories indicate progress in the development of methods for the use of soybean protein for paper sizing and for the production of artificial fibers resembling wool. With continued research many new industrial uses will be found for the soybean.

In the past chemistry has played an important role in helping the farmer to produce adequate food supplies. Perhaps in the future it may serve agriculture equally as well by finding new markets for the farmer's surplus crops.

In conclusion, I wish to express my sincere appreciation for the honor that you have conferred upon me by granting me the privilege of serving as President of our Association.

To those new members who are attending the meeting for the first time I wish to extend a cordial welcome. I would urge you to take an active interest in the work of our Association.

I am glad to have this opportunity to express our appreciation to those officers of the Association who are chiefly responsible for the success of our work. We are indebted especially to Dr. Skinner, our Secretary-Treasurer, to Mr. Lepper, Chairman of the Committee on Recommendations of Referees, to Miss Lapp, and to the referees, associate referees, and collaborators.

A marked increase in attendance at our meetings and in the number of subjects studied by the referees in recent years illustrates the continued growth of our Association. With increased interest in the development of methods for the utilization of farm crops and the recent Federal legislation providing for the control of the sale of cosmetics, new subjects will require our attention. I am confident that our Association will continue in the future to meet these new responsibilities as it has in the past.

ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

THIRD DAY

WEDNESDAY—AFTERNOON SESSION

REPORT OF EDITORIAL BOARD

By W. W. SKINNER, *Chairman*

Mr. Lepper of the Board of Editors will make a detailed statement about the affairs of *The Journal,* and in the absence of Dr. Bailey I shall make a statement about the editorial work of the Committee on *Methods of Analysis.*

Only a few years ago *The Journal* had an annual deficit, but the interest in this publication has increased to such an extent that in the last few years there has been a slight surplus annually. In 1935 the surplus was $265.49; in 1937 it was $978.45; and this year (1938) it is $800.00. The sale of *Methods of Analysis* has continued at such an increased rate that we find it necessary now to consider the reissue of the book. In the past we have revised our methods at five-year intervals, and it usually required about a year after the last session of the five-year period to complete the work.

We have, unsold, only about 500 copies of the 1935 issue. At the average rate of sale this means that before the fifth revision is ready in 1940, there will be a period when there will be no copies for sale. It was decided at a meeting of the Executive Committee that we would proceed at once in the revision work so far as that can be done prior to the meeting in 1939, and then incorporate the changes made in 1939, the purpose of course being to get the manuscript for the 1940 revision to the printer as soon after January first, 1940, as possible.

Dr. Bailey is unable to attend this meeting. It is probable that he will not be able to give a large amount of time to the 1940 revision, but it is the desire of the Committee, subject to Dr. Bailey's approval, that he continue as chairman and we arrange to have the work done by the staff.

The company that published *Principles and Practice of Agricultural Analysis* was in a poor financial condition, and the Committee learned that the officers desired to dispose of the accumulated unbound volumes of the first, second, and third editions. After some correspondance, we purchased all the unbound and a few bound copies of Volumes 1, 2, and 3 of this publication at a cost of $178.75.

Now I think, Mr. Chairman, it would be well to ask Mr. Lepper to make the report on *The Journal.*

REPORT OF EDITORIAL COMMITTEE OF *THE JOURNAL*

Your *Journal* this year has kept pace with the continued progress of the Association. The 716 pages in Volume 21 for the year of 1938 are within 28 pages of the largest volume ever issued, that is Volume 8, and that volume covers one and a half years and consists of six numbers. The number of pages this year in the section on contributed papers is 222, practically the same as that published in each of the last few years. Only normal growth is to be expected in the section devoted to the proceedings as the number of referees gradually increases with added subjects of study. However, the future development of *The Journal* as the foremost medium of papers on agricultural chemistry and related subjects will depend upon the quality and number of contributed papers. We are all interested in this future and should continue to support *The Journal* by giving it first consideration as a means of publication.

HENRY A. LEPPER, *Editor*

I think it is a great satisfaction to hear the report made by Mr. Lepper about the increased interest in *The Journal.* Through the members of the Association, through *The Journal,* and through our *Methods of Analysis,* the name of the Association has been extended widely. Subscriptions to *Methods of Analysis* and *The Journal* go to such far distant countries as China, Java, India, and Australia. This splendid reputation is a matter in which we should take a great deal of pride.

No report was given by the Committee on Quartz Plate Standardization and Normal Weight.

REPORT OF THE COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

First Adoption as Official

DOLOMITE

Dolomite is a mineral composed chiefly of carbonates of magnesium and calcium in substantially unimolal (1–1.19) proportions.

PRIMARY FERTILIZER COMPONENTS

Primary Fertilizer Components are those at present generally recognized by law as necessary to be guaranteed in fertilizers, namely: nitrogen, phosphoric acid (P_2O_5) and potash (K_2O).

SECONDARY FERTILIZER COMPONENTS

Secondary Fertilizer Components are those other than the "primary fertilizer components" that are essential to the proper growth of plants and that may be needed by some soils. Some of these components are calcium, magnesium, sulfur, manganese, copper, zinc, and boron.

BAT MANURE

Bat manure is the dry excrement from bats.

BAT GUANO

Bat guano is partially decomposed bat manure.

ANALYSIS

The word *analysis,* as applied to fertilizer, shall designate the percentage composition of the product expressed in those terms that the law requires and permits.

Second Reading as Tentative
CALCIUM NITRATE

Calcium nitrate (nitrate of lime) is a commercial product consisting chiefly of calcium nitrate, and it shall contain not less than fifteen per cent (15%) of nitrogen.

AMMONIATED SUPERPHOSPHATE

Ammoniated superphosphate is the product obtained when superphosphate is treated with ammonia or with a solution containing free ammonia and other forms of nitrogen dissolved therein.

First Reading as Tentative
SUPERPHOSPHATE

Superphosphate, 24 per cent or below, is a commercial product consisting largely of available phosphates and calcium sulfate resulting from treating ground phosphate rock with sulfuric acid. The grade that shows the available phosphoric acid should always be used as a prefix to the name.

Example: 18 per cent superphosphate.

Superphosphate, over 24 per cent, is a commercial product consisting largely of available phosphates and some calcium sulfate resulting from treating ground phosphate rock with phosphoric acid or both phosphoric acid and sulfuric acid. The grade that shows the available phosphoric acid should always be used as a prefix to the name.

Example: 45 per cent superphosphate.

or

Superphosphate is a commercial product, the phosphoric acid content of which is due chiefly to mono-calcium phosphate.

or

Superphosphate is a commercial product made from rock phosphate by substantial conversion of its phosphoric acid (P_2O_5) content into available ortho forms, with inclusions of compounds either native to the rock or produced in the manufacturing process.

(The phosphoric acid (P_2O_5) content shall be stated.)

Proposed Definition

Nitrate of Soda and Potash is a commercial product containing nitrates of sodium and potassium. It shall contain not less than fourteen per cent (14%) nitrogen (N) and fourteen per cent (14%) potash (K_2O).

L. S. WALKER, *Chairman*   H. D. HASKINS (Absent)

G. S. FRAPS   W. C. JONES

L. E. BOPST   W. H. MacINTIRE

Approved.

REPORT OF COMMITTEE ON RECOMMENDATIONS
OF REFEREES

In the report of this Committee last year a plan to broaden the collaborative work of the Association was proposed. A circular was sent to each subscriber to *The Journal* and an invitation to cooperate was extended to every interested scientific worker in the many fields of the Association's activities. Many replies were received on return coupons. Some offers duplicated those already on record but the resulting increase in collaboration seemed to justify a repetition of this service for our referees. Plans have been made to have the notice appear in the first number of *The Journal* following this meeting, and also to include a card, easily detachable from the page, for convenience in replying.

Our Association—and I think it may be said without any appearance of boasting—is outstanding in its contribution to the development of methods of analysis for agricultural and kindred products. This position has been achieved only through continued effort. As occasion has arisen the work of the Association has expanded to meet the need for analytical procedures of accuracy and dependability that progress in new fields of scientific development has shown to exist. This Committee especially appreciates the important part played by referees, associate referees, and collaborators in the success of the Association's program of work.

At this time a new opportunity for service and a new responsibility to maintain the Association's prestige have been presented to these active workers by the passage of the new Federal Food, Drug, and Cosmetic Act. It will be necessary to develop methods for new drugs and cosmetics. Work in the latter field was started last year by the addition of a refereeship on cosmetics. It is perhaps in connection with the formulation of legal standards for foods that more serious consideration by referees will be required in future development of methods. Standards of identity for some foods will include analytical requirements. The establishment of proper values in such cases will be the result of the analysis of products of authentic history. To judge compliance with standards, samples should be examined by the same methods that are used in the compilation of data for the standards. It is obvious that such circumstances denote a permanency of the analytical procedures involved. This does not mean that changes in methods are not to be considered, but it should operate as a caution against recommending changes that might be regarded as minor and appear to involve no appreciable differences in results.

Empiricism takes on a new significance. We now recognize that methods that appear, on their face, to be simple determinations of well understood constituents, as, for example, those for moisture and ash, are in reality largely empirical. Minor changes would usually involve merely going from one empirical procedure to another. It can readily be appreciated that minor changes in several successive years may finally result

in methods giving results far different from those obtained under the original directions. The adequacy of such revised methods in judging the identity of a given lot of food might be seriously challenged. In the enforcement of legal standards it is desirable to limit discussion of analytical tolerances to questions of the accuracy established for the method employed.

There is no thought in this discussion that a referee should refrain from improving methods, but study should be directed toward changes resulting in greater accuracy, precision, or economy of time. When these ends can be accomplished the adoption of new or revised methods would justify the additional studies required to correlate the results by such methods with those by the original ones to establish an interpretative basis for evaluating the results by the newer methods.

Another duty presents itself to the referees at this time, especially those on foods, that of reviewing their respective chapters with a view to perfecting present methods by revision or substitutions if it is considered necessary to make them more serviceable for use in compiling authentic data in the standardization of foods. Also all referees should begin now to consider necessary changes in their respective chapters in anticipation of the scheduled revision of *Methods of Analysis* in 1940, and to remember that collaborative results and two recommendations are required for adoption, deletion, or changes of official methods. It is becoming more and more imperative with each revision that referees consider the possibilities of uniformity and the need for cooperation to eliminate repetition of directions. Again and again methods for the determination of a constituent appear under two or more products, varying only in minor details. With little effort the referees concerned could agree to a unified procedure, so that the fundamental directions could be given in one chapter and referred to in the others. Only such additional directions as make the fundamentals of the method applicable to the specific product need be given in the cross reference. Referees should be alert to opportunities for study in new fields and should recommend the inauguration of new work where need is indicated.

Without any intent to criticize the work of the referees, it is urged that work be not postponed, as is often the case, but that it be begun as soon after appointment as possible. It is appreciated that the work is often accomplished by crowding it in with other necessary duties, but even under such circumstances it would appear that the sooner it is begun the greater the accomplishment in the aggregate. Of interest in this connection is a letter received by the Association from a chemist for a large food manufacturing firm of nation-wide reputation, who points out that less than a month before the meeting this year he was furnished samples for collaboration by five different referees. He had previously expressed his wish to cooperate, but found it a serious drain on his time to do so in

the short time available. Early attention to sending out collaborative samples would often enlist the services of more collaborators and eliminate some of the last minute rush in the preparation of reports and recommendations, which frequently leads to errors that might otherwise be avoided.

The duties of referees and associate referees, as defined in the constitution, have been elaborated upon in a report by E. M. Bailey, published in *This Journal*, **17,** 42 (1934). This report also presents practical recommendations for the preparation of referee reports. Every referee is urged to read that report. Experienced referees will find it helpful in refreshing their ideas of A.O.A.C. procedure, and new referees will find it a valuable guide.

No report of this committee would be complete without an expression of appreciation of the unselfish work—often performed at personal sacrifice—of the referees, associate referees, and collaborators.

<div align="right">HENRY A. LEPPER, Chairman</div>

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES*

By G. E. GRATTAN (Department of Agriculture, Ottawa, Canada), *Chairman;* H. A. HALVORSON, and E. L. GRIFFIN

STANDARD SOLUTIONS

It is recommended—

(1) That the method submitted by the referee for the standardization of acid solutions with borax (see p. 102) be adopted as official (first action).

(2) That the method submitted by the referee for the standardization of acid solutions with sodium carbonate (see p. 103) be adopted as official (first action).

(3) That no further study be given to the standardization of hydrochloric acid solutions by silver chloride.

(4) That collaborative work be done on the method submitted by the referee for standardization of iodine solutions.

(5) That standardization of sodium thiosulfate be studied further.

(6) That the tentative methods for the preparation and standardization of solutions of sodium hydroxide (p. 681, 1) be adopted as official (first action).

(7) That the tentative methods for the preparation and standardization of hydrochloric acid (p. 682, 5) be adopted as official (first action).

(8) That the preparation and standardization of sulfuric acid solutions be studied.

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* These recommendations, submitted by Subcommittee A, were approved by the Association. Unless otherwise given, all references are to *Methods of Analysis, A.O.A.C.,* 1935.

(9) That the preparation and standardization of potassium permanganate solutions be studied.

(10) That the preparation and standardization of silver nitrate and thiocyanate solutions be studied.

<div align="center">INSECTICIDES, FUNGICIDES, AND CAUSTIC POISONS</div>

It is recommended—

(1) That the study of methods for the analysis of pyrethrum products be continued, and that special attention be given to the effect of a preliminary treatment for the removal of acidic substances, and also to the effect of varying quantities of sulfuric acid on the decomposition of the pyrethrum during the distillation.

(2) That the study of methods for the analysis of derris and cubé products be continued, with additional collaborative work.

(3) That the lead chlorofluoride method for the determination of fluorine be studied next year.

(4) That further study be given the determination of naphthalene in poultry lice products.

(5) That collaborative work be undertaken on the phenol coefficient method (pp. 68–72, 141–146).

<div align="center">FEEDING STUFFS</div>

It is recommended—

(1) That the Associate Referee on Ash continue his study and collaborative work.

(2) That the method submitted by the associate referee for the determination of manganese in grain and stock feeds (see p. 78) be adopted as tentative and that further collaborative work be undertaken.

(3) That study be continued on the detection of starch or starchy materials as an adulterant of condensed milk products.

(4) That a study be made of methods for the detection of adulteration of cod liver oil.

(5) That preliminary work be continued on the method for the determination of small amounts of iodine in feeding stuffs.

(6) That the study of the method for the determination of calcium in mineral feeds be continued and additional collaborative work be undertaken.

(7) That studies on the determination of fat in fish meal be continued.

(8) That the Peterson-Hughes method for the determination of carotene be adopted as tentative, and that the spectrophotometer be used on the 0.1 per cent potassium dichromate reference standard.

(9) That the potassium dichromate standard be checked by several chemists against pure beta carotene and the best conditions for accurate application be established before further collaborative work is done involving the use of this reference standard.

(10) That the study of the application to the carotene determination of the neutral wedge photometer and the photoelectric colorimeter be continued.

(11) That study be made on the application of a qualitative procedure for the determination of carotene.

(12) That the associate referee study the suggestions made in the two contributed papers on the determination of carotene given at the Annual Meeting of this Association.

(13) That the study of devising standard methods for sampling be continued.

(14) That the vacuum oven and the electric air oven methods for the determination of moisture be further studied as a group with a view to unification.

(15) That the work of correlating the moisture methods of the Association be continued.

(16) That the study of the determination of lactose be continued for another year and that consideration be given to a correction factor whereby small commercial yeast cakes may be used and to further work on the use of alcohol for elimination of interference from peanut meal and low-grade tankage.

(17) That studies on hydrocyanic acid be continued.

(18) That the general referee consider the need for the development of a method for the determination of castor seed in feeding stuffs.

(19) That the method for Vitamin D assay (p. 351, 55) be revised as recommended by the associate referee (see p. 80) and study be continued.

FERTILIZERS

It is recommended—

(1) That the methods outlined by the associate referee for the determination of calcium be studied further and that collaborative work be undertaken; also that the tentative method for the determination of calcium oxide in mineral feeds be considered (p. 347, 44).

(2) That further study be made of the methods for the determination of sulfur.

(3) That the methods outlined by the associate referee for the determination of copper be studied collaboratively and that other methods be investigated.

(4) That the method outlined by the associate referee for the determination of zinc be studied collaboratively.

(5) That the study of the use of a factor weight or weights in the determination of potash in fertilizers be continued.

(6) That the official barium chloride method for the determination of potash (31, 45, 46, 47) be deleted (final action).

(7) That the study of methods for the recovery of platinum be continued.

(8) That a study be made of the determination of potash by "dissolving out the potassium chloroplatinate and reweighing when the filtration is made on a glass sinter or asbestos padded Gooch," in place of "by filtration after ignition and solution," when platinum or silica dishes are used.

(9) That further study be made of the need for providing additional platinum solution concentrations.

(10) That a collaborative study be made of some modification of the present official method for the determination of potash to prevent foaming during the boiling of the sample.

(11) That a collaborative study be made of the degree of fineness of grinding, with a view to elimination of the errors resulting from the non-uniformity of the 2.5 gram samples weighed out for the official potash determination.

(12) That the studies concerned with the solvent action of acid alcohol on potassium chloroplatinate be continued.

(13) That the Associate Referee on Potash consider the data presented at the Annual Meeting in the paper by H. R. Allen (see p. 162).

(14) That a collaborative study be made of the beaker method in comparison with other methods for the determination of water-insoluble nitrogen.

(15) That the use of potassium persulfate along with mercury as a catalyst in the determination of total nitrogen be studied on such materials as Canadian fish meal, meat scraps, and coconut meals.

(16) That in the Kjeldahl method for the determination of organic and ammoniacal nitrogen (p. 23, 19), the last sentence in par. 19(g) be changed as suggested by the associate referee (see p. 70) (first action).

(17) That the reduced iron method for the determination of nitrate and ammoniacal nitrogen in mixed fertilizers or nitrate salts (p. 26, 31) be deleted (final action).

(18) That the official gravimetric method for the determination of water-soluble phosphoric acid (p. 21, 13) be modified as suggested by the associate referee (see p. 70).

(19) That the official method for the determination of citrate-insoluble phosphoric acid (p. 21, 15) be changed as suggested by the associate referee (see p. 70).

(20) That the Associate Referee on Phosphoric Acid give further consideration to the method proposed by MacIntire, Shaw, and Hardin for the determination of available phosphoric acid.

(21) That the method for the determination of magnesia in water-soluble compounds (*This Journal*, 21, 77) be adopted as official (first action).

(22) That the Bartlett-Tobey method for the determination of magnesium be adopted as tentative (see p. 71).

(23) That the Shuey volumetric method and other modifications of the present method for the determination of acid-soluble magnesia be studied.

(24) That the study of methods for the determination of active magnesia in mixed fertilizers be continued.

(25) That the volumetric method for the determination of acid-soluble manganese in fertilizers and manganese salts (*This Journal*, 21, 292), with minor changes as noted by the referee (see p. 71), be adopted as tentative.

(26) That the colorimetric modification of the method for the determination of acid-soluble manganese be studied further.

(27) That the methyl red indicator in the tentative method for the determination of acid- and base-forming quality of fertilizers (p. 34, 55) be changed as suggested by the associate referee (see p. 71).

(28) That in the same method, under "Determination," line 17, the direction, "add 10 drops, etc.," be changed to "add 0.4 cc. of the mixed indicator."

(29) That the same method be modified by making optional the use of a filter paper cone for the prevention of spattering.

(30) That in the same method the elimination of water-insoluble material coarser than 20 mesh before the method is applied be studied further.

(31) That the basicity of phosphate rock and other factors that affect the method be studied further.

(32) That the recommendation of the Referee on Fertilizers that an associate referee be appointed to work in cooperation with the Bureau of Standards on the testing of volumetric apparatus and weights and to recommend methods for discouraging the use of such apparatus and weights that are too inaccurate be deferred for further study.

SOILS AND LIMING MATERIALS

It is recommended—

(1) That studies of the function of ammonia in the alcohol wash used in the determination of base exchange capacity be continued.

(2) That further study be made of the calcium peroxide method for the determination of fluorine in soils and that comparative data be obtained on the fluorine content of soil by fusion with sodium hydroxide or potassium hydroxide in a nickel crucible as compared to the calcium peroxide procedure.

(3) That the ammonium chloride steam distillation procedure for the evaluation of limestone availability be studied further in relation to soil carbonate reactions in pot experiments.

(4) That studies of the factors that influence the determination of *p*H value of soils in arid and semi-arid regions be continued.

(5) That work be continued on the determination of selenium in soils.

PLANTS

It is recommended—

(1) That Volumetric Method II for the determination of chlorine (p. 131, **38**) as modified by the associate referee (see p. 72) be made official (first action) and that work be discontinued.

(2) That the Associate Referee on Chlorine prepare a statement of the limitations of the applicability of the present official (alkaline ignition) method to be made a part of the method (see p. 72).

(3) That collaborative work be done on the determination of iodine.

(4) That methods for the determination of reducing sugars, sucrose, and starch be studied further.

(5) That studies be begun on methods for the determination of fructosans.

(6) That studies be begun on methods for the determination of glucose and fructose.

(7) That the studies of inulin be continued.

(8) That the study of hydrocyanic acid in plants be continued and collaborative work initiated.

(9) That the study of the Hick method for the determination of potassium in the presence of many other elements be continued and collaborative work undertaken.

ENZYMES

It is recommended—

(1) That an associate referee be appointed under drugs to study methods for the determination of pepsin.

(2) That the tentative method for the determination of the proteolytic activity of papain (*This Journal*, **21**, 97) be studied further.

(3) That further study be given the method of Balls and Hoover for the clotting of milk by papain, *J. Biol. Chem.*, **121**, 737 (1937).

LIGNIN

It is recommended that further study be given to the determination of lignin in plants.

PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

It is recommended—

(1) That the standard methods of the American Society for Testing Materials for testing skinning and alkali resistance of varnishes (D154-38) be studied.

(2) That the study of the methods of testing abrasion resistance and hardness of varnish films be continued.

(3) That study of a method for soap resistance of varnish be made and that a study be made with a view to revising the present method of testing elasticity or toughness of varnish films in order to make execution of the method less tedious.

(4) That the study of the accelerated weathering of paints be continued.

It is recommended—

(1) That study of the spectrophotometric determination of vitamin A be continued.

(2) That further studies be made of the feeding of skim milk or whole non-vitamin D milk with the reference oil in order to determine whether the reference oil and a quantity of milk, equal to that of the vitamin D milk being assayed, should be used as a reference standard instead of the reference oil alone.

(3) That study of biological methods for the determination of vitamin B_1 in foods be continued.

(4) That investigational and collaborative work be continued on biological methods for the determination of vitamin D carriers and that the text of the tentative method be revised as suggested by the referee (see p. 80).

(5) That an Associate Referee on Vitamin K be appointed.

(6) That an Associate Referee on Riboflavin be appointed.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES*

By L. B. BROUGHTON (University of Maryland, College Park, Md.), *Chairman;* H. J. FISHER and A. E. PAUL

NAVAL STORES

It is recommended that the subject of naval stores be continued.

RADIOACTIVITY

It is recommended that the subjects of radioactivity, quantum counter and gamma ray scope be continued.

COSMETICS

It is recommended—

(1) That in view of legislative trends in the regulation of cosmetics the referee consider the need for enlarging the activities of the Association in this field and recommend such studies as appear necessary, to be begun this year if desirable.

(2) That the referee recommend the appointment of associate referees to assist in such work when needed.

DRUGS

MICROCHEMICAL TESTS FOR ALKALOIDS

It is recommended—

(1) That the tests proposed by the associate referee be adopted as tentative (see p. 88).

(2) That berberine be further studied, and that the additional products recommended by the associate referee (coniine, cosysine, phenacaine, stovaine) also be studied.

(3) That the status of the tentative microchemical tests for the alkaloids named by the referee (see p. 89) be advanced to official (first action).

MICROCHEMICAL TESTS FOR SYNTHETICS

It is recommended—

(1) That the microchemical methods proposed by the associate referee for the identification of diallylbarbituric acid, mandelic acid, and sulfanilamide be adopted as tentative (see p. 89).

(2) That the subject be continued for the study of other important synthetics, including plasmochine, benzedrine, para-phenylenediamine, and para-toluenediamine.

(3) That the changes in reagents for synthetics suggested by the referee be adopted (see p. 90).

(4) That the status of the tentative microchemical tests for the drugs named by the referee (see p. 90) be advanced to official (first action).

HYPOPHOSPHITES

It is recommended that the method proposed by the associate referee be adopted as tentative (see p. 90), and that the subject be discontinued.

DAPHNIA METHODS

It is recommended that study of this subject be continued.

HEXYLRESORCINOL

It is recommended that the method published last year (*This Journal*, 21, 536) be adopted as tentative, and that the subject be closed.

ERGOT ALKALOIDS

It is recommended that study of this subject be continued.

NITROGLYCERIN

In view of the difficulties experienced in devising an accurate method applicable in complex mixtures, it is recommended that the study of this subject be discontinued.

GUAIACOL

It is recommended—

(1) That the associate referee's adaptation of the method for the alkoxyl groups (see p. 100) to the determination of guaiacol be adopted as a tentative method.

(2) That the topic be continued for the study of methods for the determination of guaiacol in mixtures.

BIOLOGICAL TESTING

It is recommended that this subject be continued.

IODINE OINTMENT

It is recommended—

(1) That adoption of the method for the determination of free iodine (21, 94) as official be deferred until methods for the other forms of iodine are also available.

(2) That the study of methods for inorganically combined iodine, which was started by the associate referee in 1936, be resumed.

ACETYLSALICYLIC ACID ACETOPHENETIDIN AND CAFFEINE

It is recommended that the method proposed by the associate referee for the separation of acetylsalicylic acid, acetophenetidin, and caffeine, be adopted as tentative (see p. 91) and that the subject be closed.

GUMS

It is recommended that the qualitative tests for gums proposed by the associate referee be adopted as tentative (see p. 92) and that the subject be closed.

THEOBROMINE AND THEOBROMINE CALCIUM TABLETS

It is recommended—

(1) That the present tentative method (p. 590, 137) be retained in that status.

(2) That the method proposed by the associate referee be tentatively adopted as an alternative method (see p. 94).

(3) That the subject be closed.

CHLOROBUTANOL

It is recommended that the method proposed by the associate referee for the determination of chlorobutanol and chlorobutanol in solutions be adopted as tentative (see p. 95) and that the subject be closed.

ACETYLSALICYLIC ACID AND PHENOLPHTHALEIN TABLETS

It is recommended that the method proposed by the associate referee be adopted as tentative (see p. 95), and that the subject be closed.

AMINOPYRINE AND PHENOBARBITAL TABLETS

It is recommended that the subject be continued for study of the separation of these drugs in mixtures.

ELIXIR OF TERPIN HYDRATE AND CODEINE

It is recommended that this subject be continued.

EMULSIONS

It is recommended that the method submitted by the associate referee be adopted as tentative (see p. 96), and that the subject be closed.

CITRINE OINTMENT

It is recommended that the method submitted by the associate referee for the determination of mercury in citrine ointment be adopted as tentative (see p. 96), and that the subject be closed.

RHUBARB AND RHAPONTICUM

It is recommended that this subject be continued.

THEOPHYLLINE SODIUM SALICYLATE

It is recommended that this subject be continued.

SULFANILAMIDE

It is recommended that the method described in Part II of the associate referee's report be adopted as tentative (see p. 97), and that the topic be closed.

MANDELIC ACID

It is recommended—

(1) That the method proposed by the associate referee for tablets be adopted as tentative (see p. 98).

(2) That the method proposed by the associate referee for the determination of mandelic acid in liquid preparations be adopted as tentative and include the suggestions of the Committee (see p. 98).

(3) That the qualitative tests for mandelic acid proposed by the associate referee be adopted as tentative and include the changes suggested by the Committee (see p. 98).

(4) That this subject be discontinued.

CHANGES IN METHODS

CAMPHOR

It is recommended (first action) that the statement, "Not applicable to synthetic camphor," be inserted in parentheses between the title and the text of the official method (p. 560, 51).

BISMUTH COMPOUNDS IN TABLETS

It is recommended—

(1) That the tentative method (p. 592, 143) be amended by the addition of directions for preparation of sample (see p. 98).

(2) That the statement "Lead absent" following the title "Bismuth Compounds" be changed to read, "Not applicable in the presence of lead compounds but applicable in the presence of cerium salts."

COCAINE

It is recommended—

(1) That the last paragraph in Method II (p. 576, 97) be transposed

and constitute a second paragraph in Sec. **96**, p. 576, and that the status of this paragraph be advanced to that of official (first action).

REAGENTS FOR MICROCHEMICAL TESTS

It is recommended that the changes in the reagents suggested by the referee be adopted (see pp. 90, 99).

CHLOROFORM IN MIXTURES

It is recommended that the changes in the tentative method for the determination of chloroform in mixtures suggested by the author of a special report presented at the 1938 meeting of the Association be adopted as tentative (see p. 99).

BARBITAL AND PHENOBARBITAL

It is recommended that the official method (p. 582, **112**) be amended (first action) as suggested by the associate referee (see p. 99).

PHENOLPHTHALEIN

It is recommended that the methods for the determination of phenolphthalein (p. 569, **76**, **78**) be amended as suggested (see p. 99).

THYMOL

It is recommended that the method for the determination of thymol (p. 571, **84**) be adopted as official (final action).

CHANGES IN STATUS OF METHODS

The tentative methods for the assay of the drugs recommended by the referee were adopted as official (first action) (see p. 100).

NEW SUBJECTS

It is recommended that the following subjects be studied:

(1) Acetophenetidin, acetylsalicylic acid, and salol in mixtures.
(2) Arecoline hydrobromide (particularly assay of tablets).
(3) Benzedrine.
(4) Hydroxyquinoline sulfate.
(5) Ipecac and opium powder (Dover's powder).
(6) Yellow oxide of mercury ointment.
(7) Acetanilid and salol (separation).
(8) Physostigmine salicylate.
(9) Pepsin.
(10) Plasmochine.
(11) Microchemical tests (coniine, cosysine, phenacaine, stovaine, plasmochine, benzedrine, para-phenylenediamine, and para-toluenediamine.)
(12) Purification of caffeine in plant extractives.
(13) Nicotinic acid.
(14) Epedrine in jellies.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES*

By J. O. CLARKE (U. S. Food and Drug Administration,
Chicago, Ill.), *Chairman;* G. G. FRARY, and W. B. WHITE

CANNED FOODS

It is recommended—

(1) That the method for determination of alcohol-insoluble solids in canned peas (*This Journal*, 21, 89), with minor clarifying changes, be adopted as official (final action) (see p. 87).

(2) That the method for determination of chlorides in tomato juice (*This Journal*, 20, 78), with minor clarifying changes, be made official (final action) (see p. 88).

(3) That the official method for preparation of sample (p. 497, 2) be extended to include canned fruit and be clarified by requiring tilting of the sieve during draining, and turning of all pieces to permit drainage of cups or cavities.

(4) That studies of methods for quality factors and fill of containers be continued.

(5) That collaborative studies be conducted on the tentative method for total solids in tomato products (p. 499, 16) with a view to its adoption as an official method.

DAIRY PRODUCTS

It is recommended—

(1) That studies of methods for the detection of neutralizers in dairy products be continued, and that particular attention be given to the ratio between titratable acidity and lactic acid.

(2) That methods for the determination of lactic acid in dried milk be further studied.

(3) That the Associate Referee on Malted Milk study the application of the official method for fat in dried milk (p. 282, 72) to malted milk with a view to final adoption of a single method for both products.

(4) That methods for the separation of fat in dairy products (except cheese) for the determination of fat constants be further studied.

(5) That the Associate Referee on Cheese study methods of isolating fat from cheese for the determination of fat properties and constants, giving special attention to such treatments as promise a minimum change in the properties of the fat.

(6) That studies of methods for the determination of casein in malted milk be continued.

(7) That studies be continued on the development of a satisfactory stirrer method for preparation of butter samples.

(8) That the study of rapid methods for determining the degree of pasteurization in milk and cream be continued.

(9) That an associate referee be appointed to study methods for examining butter for the purpose of detecting the use of under-pasteurized cream.

(10) That studies on mounting media in the microscopic method for the identification of malted milk be discontinued.

(11) That studies of alternative methods for detecting gelatin in milk and cream be discontinued.

(12) That studies of methods for the determination of casein be discontinued.

(13) That further studies on the determination of citric acid in milk be postponed until the completion of studies on the determination of citric acid in fruit.

(14) That studies on methods for the clarification of milk for the optical determination of lactose be continued and broadened to include correction for volume of the precipitate.

(15) That studies on methods for the detection of decomposition in dairy products be continued.

(16) That the method for estimation of mold mycelia in butter suggested by the associate referee (*This Journal*, 20, 93) be adopted as tentative (see p. 76) and studied collaboratively.

(17) That studies be continued on methods to distinguish between products made from cow's milk and those made from the milk of other animals.

(18) That study of methods of analysis of frozen desserts be inaugurated.

EGGS AND EGG PRODUCTS

It is recommended—

(1) That studies of methods for determining water-soluble and crude albumin in dried eggs be discontinued.

(2) That work on chemical methods for detecting decomposition in eggs be continued.

(3) That studies of methods for the determination of cholesterol and fat be continued.

(4) That the official method for determination of chlorine in eggs (p. 301, 16 and 17) be changed (first action) to provide for the substitution of the official volumetric procedure for the official gravimetric procedure in the final measurement of chlorine (see p. 77).

(5) That the method, official (first action) for determination of dextrose and sucrose (301, 18 and 19), be modified as suggested by the referee (see p. 77).

(6) That studies on methods for the determination of glycerol be continued.

FISH AND OTHER MARINE PRODUCTS

It is recommended—

(1) That the tentative methods for determination of ash, salt, and total nitrogen (*This Journal*, 21, 86) be studied collaboratively.

(2) That further study be made of methods for the determination of ether extract and total solids.

GUMS IN FOODS

It is recommended that studies on methods for determination of gums in foods be continued.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That the method for determination of nitrates in meat products suggested by the referee be adopted as tentative (see p. 82) in place of the phenoldisulfonic acid method (p. 356, 14 and 15).

(2) That studies of methods for the detection of dried skim milk in meat products be continued.

(3) That the change in the manner of using the indicator in the method for the determination of coagulable nitrogen (p. 360, 28) suggested by the referee be incorporated in the method (see p. 83).

(4) That the present tentative methods for determination of copper and zinc in gelatin (p. 368, 64 and 65) be dropped and the methods suggested by the referee be adopted as tentative (see p. 84).

METALS IN FOODS

It is recommended—

(1) That studies be continued on methods of sample preparation of those products wherein the arsenic is tenaciously held.

(2) That the iodine titration, gold or silver sol, and the molybdenum blue colorimetric methods for the determination of arsenic be further studied as possible substitutes for the Gutzeit method.

(3) That in the studies of methods for the determination of antimony and of arsenic special attention be given to the separation of micro quantities of these elements occurring simultaneously in organic or biological material.

(4) That studies on micro methods for the determination of copper be continued.

(5) That collaborative studies on methods for the determination of fluorine in phosphates and baking powder be continued, and that special attention be given to methods of sample preparation for organic materials.

(6) That the colorimetric dithizone and the electrolytic methods for the rapid determination of lead on apples and pears (p. 391, 30–33) be simplified as suggested by the associate referee (see p. 85) and that the simplified method be made official (final action).

(7) That studies of methods for the determination of lead be continued, with special reference given to oils and baking powders, and to the simplification of methods for removing interfering substances.

(8) That studies on methods for the determination of mercury be continued.

(9) That the method for selenium proposed by the associate referee (see p. 85) be adopted as tentative and subjected to further study.

(10) That studies on micro methods for the determination of zinc be continued.

(11) That the method for determining hydrocyanic acid developed by the associate referee be subjected to further study.

OILS, FATS, AND WAXES

It is recommended—

(1) That the modified Kaufmann method for determining the thiocyanogen number of fats and oils (*This Journal*, 21, 87) be studied collaboratively with a view to its adoption as official.

(2) That the official method for determining free fatty acids (p. 417, 30) be dropped (final action).

(3) That the N.C.P.A. methods for determining free fatty acids in crude and in refined oils (*This Journal*, 21, 88) be made official (final action).

(4) That the refractometric method for the determination of oil in flaxseed (*This Journal*, 20, 74) be made official (final action).

(5) That studies be made on the application of the associate referee's method for the determination of oil in flaxseed to other commercially important oil seeds.

(6) That studies on the Polenski method be continued.

(7) That the specifications for the titer thermometer (p. 408, 15) be amended in accordance with the revised Bureau of Standards specifications.

SPICES AND OTHER CONDIMENTS

It is recommended—

(1) That the tentative method for the determination of volatile oil in spices (p. 447, 16) be made official for the same determination in marjoram and sage (first action), and that the method be further studied with respect to other spices.

(2) That the method proposed by the associate referee for the determination of ash in vinegar (*This Journal*, 21, 89) and adopted as official (first action) last year, be adopted as official (final action).

(3) That the Referee on Vinegar study methods for the determination of total phosphoric acid.

(4) That the official method for the determination of solids in vinegar be studied, especially with reference to its application to vinegars high in solids, such as malt vinegar.

(5) That methods for the detection of caramel in vinegar be studied.

MICROBIOLOGICAL METHODS

It is recommended that studies be continued on the microbiological examination of canned vegetables, canned tomatoes and fruits, canned fishery products, canned meats, and sugar; and that similar studies be undertaken on eggs and egg products.

REPORT OF SUBCOMMITTEE D ON RECOMMENDATIONS OF REFEREES*

By J. A. LeCLERC (Bureau of Chemistry and Soils, Washington, D. C.), *Chairman;* J. W. SALE and W. C. JONES⁻

SUGARS AND SUGAR PRODUCTS

It is recommended—

(1) That the vacuum drying method adopted by the International Commission for Uniform Methods of Sugar Analysis (*This Journal,* 21, 89) be made official (final action).

(2) That the International Scale of Refractive Indices of Sucrose Solutions at 20° C., 1936, be adopted as official (final action).

(3) That the International Temperature Correction Table, 1936, be adopted as official (final action).

(4) That the official method of Wein for the determination of maltose (p. 484, 54–55) be dropped (final action).

(5) That the work on methods for determining acetyl-methyl carbinol and diacetyl in food products be continued.

(6) That the work on methods for determining the so-called unfermentable sugars of molasses be continued.

(7) That the study of maple flavor concentrates and imitations be continued.

(8) That studies on the determination of moisture in honey be continued.

(9) That the work on refractive indices of invert sugar solutions and the change in refractive indices with change of temperature in such products as invert sugar solutions, table sirups, etc., be continued.

(10) That the study of polariscopic methods be continued along the lines covered by the recommendations made and approved in 1931, 1932, and 1933.

(11) That study of chemical methods for reducing sugars be continued.

(12) That study of drying, densimetric, and refractometric methods be continued.

(13) That an Associate Referee on Sucrose and Ash in Molasses be appointed.

It is recommended—

(1) That the statement on p. 506, 14(c), "0.0001 mg of N as NO_2," be changed to read: "0.0001 mg of N" (final action).

(2) That the determination of boron in waters be further studied.

(3) That the work on effervescent salts be continued.

(4) That the study of the thorium nitrate method for the determination of fluorine in water, described in the referee's report, be further studied.

ALCOHOLIC BEVERAGES

It is recommended—

(1) That the pressure air method for the determination of carbon dioxide in beer be adopted as tentative (see p. 73) and be further studied collaboratively with the object of making it official.

(2) That the Associate Referee on Beer study collaboratively the following tentative methods relating to beer (Chap. XIV): (a) Extract in original wort; (b) real degree of fermentation; (c) total acid; (d) dextrin; (e) direct polarization; (f) pasteurization, and (g) chlorides, and that H-ion concentration also be studied.

(3) That methods for the determination of heavy metals (Fe, Cu, Pb) As, and F be studied (as recommended last year).

(4) That the viscometric method outlined by the associate referee last year for the determination of the proteolytic activity of malt (*This Journal*, 21, 160) and the edestin titration method (*Wochschr. Brau.*, 53, 297 (1936)) be further studied.

(5) That the vacuum method for the determination of moisture in flour (p. 206, 2) be studied as to its applicability to the determination of moisture in malt adjuncts (p. 161, 53).

(6) That a special study be made of methods for the determination of fat that will be applicable to corn grits and brewers' rice and flakes.

(7) That a study of the method for determining the extract in malt adjuncts (p. 161, 35) be made and that consideration be given to the suggestion to use a portion of the malt in the boiling operations.

(8) That special study be made of the diastatic activity of malt.

(9) That the study of methods for the detection of adulteration of distilled spirits be continued.

(10) That the collaborative study of sulfur dioxide in beer and ale be continued and also be extended to include this same determination in wines.

(11) That further collaborative work be done on the tentative methods for the determination of benzaldehyde (p. 183, 55), volatile esters (p. 181, 46), and gamma undecalactone (p. 181, 47) in cordials.

(12) That the study of the saponification of esters with lead acetate be dropped.

(13) That the distillation procedures for volatile acids in wines (p. 166, 23, 24) be studied further with a view to eliminating chance errors and that the modification described in the associate referee's report or some other modification of the Peynaud procedure be tested further.

(14) That the sulfite method for the determination of aldehydes in whiskey and other potable spirits be made tentative and that work on it be continued with a view to making it official.

(15) That a study on the determination of total sulfur in wines be conducted.

(16) That the evaporation method described in the associate referee's report and Method II (p. 167, 24) for the determination of volatile acids in wines be further studied to determine their applicability to distilled spirits and that the cause of the slight loss resulting from the use of Method II be investigated.

(17) That the procedure for the quantitative determination of methanol in distilled spirits by the use of the neutral wedge photometer described in the associate referee's report be subjected to collaborative study for possible further improvement and simplification.

FOOD PRESERVATIVES AND SWEETENERS

It is recommended—

(1) That further work on Special Method II (p. 435, 15) for the determination of saccharine in non-alcoholic beverages as to its applicability to apple butter be discontinued.

(2) That the work on the Illing method for the determination of benzoate of soda, *Analyst*, 57, 224 (1932), which was found to be suitable for sausage, be continued with respect to its suitability for other food products.

(3) That further studies based on the work of Tortelli and Piazza be made on the qualitative test for saccharin (p. 434, 13).

COLORING MATTERS IN FOODS

It is recommended—

(1) That collaborative work be continued in estimating ponceau SX and ponceau 3R.

(2) That investigational work be continued on the quantitative estimation of sunset yellow FCF in the presence of tartrazine.

(3) That investigational work be continued in separating and estimating quantitatively mixtures of light green SF yellowish, brilliant blue FCF, and fast green FCF.

FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That the study of soluble solids be dropped.

(2) That the study of electrometric titration be continued.

(3) That the changes in the methods for the analysis of preserves and jams suggested by the referee be adopted as official (first action) (see p. 78).

(4) That the official method for the determination of phosphoric acid (P_2O_5) in wines (p. 166, 19) be adopted as tentative for the same determination in fruits and fruit products (see p. 78).

(5) That the study of methods for the determination of inactive malic, isocitric, and lactic acids be continued.

(6) That the application to fruits and fruit products of the colorimetric method for lactic acid in dried milk be studied (*This Journal*, 20, 605).

(7) That the study of volumetric and colorimetric methods for the determination of phosphoric acid (P_2O_5) in jams, jellies, and other fruit products be continued.

(8) That the effect of slow oxidation on the yield of pentabrom acetone in the determination of citric acid be studied.

(9) That the study of polarimetric methods for jams and jellies and preserves be continued.

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That work on the determination of glycerol, vanillin, and coumarin in imitation vanilla be continued.

(2) That the chemical method described in the associate referee's report for detemination of isopropyl alcohol be studied further collaboratively and that it be applied to mixtures containing essential oils.

(3) That the spectrophotometric method for the determination of coumarin in imitation vanilla be subjected to collaborative study.

(4) That the method presented by Wilson for the quantitative determination of beta-ionone be studied collaboratively.

(5) That the referee study the possible application of automatic extraction to the determination of vanillin and coumarin in vanilla extract.

(6) That the referee study the application of the spectrophotometer to the present colorimetric methods found in the chapters on Flavors and Non-alcoholic Beverages.

CACAO PRODUCTS

It is recommended—

(1) That further collaborative work be done on the pectic acid method for the quantitative determination of shell in cacao products (*This Journal*, 20, 417; 21, 441).

(2) That work be done on the determination of lecithin in cacao products.

(3) That collaborative work be done on the method for the determination of milk protein described in the referee's report.

BAKING POWDER

It is recommended that the method described in the referee's report for the determination of free tartaric acid (direct determination), cream of tartar, and total tartaric acid be adopted as official (first action).

CEREAL FOODS

It is recommended—

(1) That the official (first action) method for sampling bread be made official (final action).

(2) That the official (first action) method for the collection and sampling of macaroni products be made official (final action).

(3) That further study be made of the tentative magnesium-acetate method of ashing (*This Journal*, **20**, 69).

(4) That the statement on p. 216, **38(c)**, "0.0001 mg of N as nitrite," be changed to read "0.0001 mg of N" (final action).

(5) That study be undertaken to develop a rapid method for the determination of starch in flour, and that further study be given to the improvement of polarimetric methods for starch determination in both raw and cooked cereal foods.

(6) That collaborative study be made to determine reducing and non-reducing sugars in flour.

(7) That the tentative method for the determination of acidity of water extract of flour be dropped (p. 208, 13).

(8) That the method for fat-acidity of flour proposed in the associate referee's report be adopted as tentative (see p. 75), and that further study be made of the methods described in the associate referee's report for the determination of acidity in flour and other cereal products.

(9) That the associate referee continue his studies on the baking test for soft wheat flour.

(10) That the study of the proteolytic enzymes of flour be continued.

(11) That the associate referee continue the study of methods for the determination of ergot in rye flour.

(12) That study be continued to develop a method for determining sodium chloride-free ash in macaroni and baked products.

(13) That work be continued on methods for the determination of soybean flour in cereal products.

(14) That the study of methods for the identification of the nature of the raw materials used in the manufacture of macaroni be discontinued for the present.

(15) That the associate referee continue his studies on whole wheat flour, giving special attention to the determination of cellulose as an index of the whole wheat content of cereal products.

(16) That further study be made of methods for the determination of the ash of the original flour used in phosphated and self-rising flours, especially of old self-rising flours, with special reference given to: (a) analysis for sodium chloride content of self-rising flour and of the ash of the extracted flours, and (b) study of means other than extraction with carbon tetrachloride for 100 per cent separation of the sodium chloride from old self-rising flours.

(17) That the study of methods for the determination of chlorine in bleached flour fat be continued.

(18) That the study of the methods for the determination of benzoyl peroxide in flour be continued.

(19) That the method for the measurement of carotinoid pigments in flour given in *This Journal*, 21, 339, be studied with a view to the substitution of water-saturated normal butyl alcohol for the Varsol alcohol mixture.

(20) That the associate referee continue his studies on H-ion concentration of flour.

(21) That the tentative citric acid method (p. 224, 55), the so-called "fat" procedure (p. 222, 54) and the lactose method given in the associate referee's report for the calculation of milk solids in bread be further studied.

(22) That the tentative method as modified by the associate referee for the determination of extract soluble in cold water be adopted as official (first action) and that further collaborative study be made with flour and other cereals and cereal products.

(23) That the method for the determination of carbon dioxide in self-rising flour be further studied collaboratively.

(24) That the suggestion made in 1936 regarding the sterol content of cereals be repeated.

(25) That further collaborative work on the measurement of flour and bread color by the N. A. colorimeter be dropped, and that study be continued on the sampling and preparation of flour and bread for color measurements in the photoelectric cell method based on reflectance.

(26) That an associate referee be appointed to study the application of the methods under wheat flour (determination of water, ash, protein, fat and crude fiber) to corn meal, corn flour, and corn starch, including fat in brewer's grits, and flakes; to rolled oats, oatmeal, and oat flour; to rye flour and buckwheat flour; to barley malt, barley flour, rice flour and fat in brewer's rice.

(27) That an associate referee be appointed to study the application of methods under bread (determination of ash and protein) to such baked products as crackers, cookies, and cakes.

(28) That the method for the determination of apparent viscosity of flour (*This Journal*, 20, 380) be adopted as official (final action).

(29) That a method for the estimation of the butterfat content of bread, based on the direct saponification and distillation of the bread, without extraction of the fat, be developed.

MICROCHEMICAL METHODS

It is recommended—

(1) That the microchemical method proposed by the referee for the determination of methoxyl be adopted as tentative (see p. 100), and that further study be made of this procedure.

(2) That the referee make a study of micro methods applicable to the work of this Association.

CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FIFTY-FOURTH ANNUAL MEETING, NOVEMBER 14, 15, AND 16, 1938*

I. SOILS

No additions, deletion, or other changes.

II. FERTILIZERS

(1) The official barium chloride method for the determination of potash (p. 31, 45, 46, 47) was deleted (final action).

(2) The last sentence in par. 19(g) of the official Kjeldahl method for the determination of organic and ammoniacal nitrogen (p. 23, 19) was changed to read as follows: "A soln having a sp. gr. of 1.36 or higher may be used" (first action).

(3) The reduced iron method for the determination of nitrate and ammoniacal nitrogen in mixed fertilizers or nitrate salts (p. 26, 31) was deleted (final action).

(4) In the official gravimetric method for the determination of water-soluble phosphoric acid (p. 21, 13), the first sentence was changed (first action) to read as follows:

Place 1 g of the sample on a 9 cm filter and wash with successive small portions of H_2O until the filtrate measures about 250 cc. Allow each portion of the wash water to pass through the filter before adding more, and wash with suction if the washing would not otherwise be complete within 1 hour.

(5) In the official method for the determination of citrate-insoluble phosphoric acid (p. 22, 16(a)) lines 1–8 were changed to read as follows:

After washing out the water-soluble P_2O_5, 13, transfer the filter and residue, within a period not to exceed an hour, to a 250 cc flask containing 100 cc of the NH_4 citrate soln previously heated to 65° in a water bath. Close the flask lightly with a smooth rubber stopper and shake vigorously until the filter paper is reduced to a pulp, relieving the pressure by momentarily removing the stopper. Loosely stopper the flask to prevent evaporation and return it to the bath. Maintain the contents of the flask at exactly 65°, keeping the level of the H_2O in the bath above that of the citrate soln in the flask. Shake the flask every 5 min.

(6) The tentative method for the determination of magnesia in water-soluble compounds (*This Journal*, 21, 77) was adopted as official (first action) under the title "Magnesia in Water-Soluble Compounds (Applicable to Sulfate of Potash Magnesia, Sulfate of Magnesia, and Kieserite)."

(7) The following method (Bartlett-Tobey) for the determination of magnesium was adopted as tentative.

MAGNESIUM

Weigh 2.5 g of fertilizer into a 250 cc volumetric flask, add 30 cc of HNO_3 and 10 cc of HCl, and boil for 30 min. Cool, make to volume, mix, filter through a dry filter paper, and transfer a 100 cc aliquot to a 400 cc beaker. Add a few drops of methyl red. Add NH_4OH until the soln is yellow, then HCl until barely pink. Add 15 cc of a saturated soln of NH_4 oxalate, adjust the soln to pH 5.0 (a faint pink color) by the addition of HCl (1 +4), or NH_4OH (1 +4), boil for a few minutes, cool, and again adjust the reaction to pH 5.0, adding more methyl red if necessary. Stir thoroughly and allow the soln to stand until the precipitate settles. Filter through a 11 cm filter paper fine enough to retain Ca oxalate and wash 10 times with hot H_2O. To the filtrate add 2 cc of 10% HCl and evaporate to a volume of approximately 100 cc. Add 5 cc of a 10% Na citrate soln and enough NH_4OH to make the soln alkaline. (Blue with bromothymol blue.) If the fertilizer does not contain soluble phosphoric acid, add 5 cc. of a 10% soln of $(NH_4)_2HPO_4$. Stir vigorously until the precipitation is completed. Add 15 cc of NH_4OH and allow to stand at least 2 hours, stirring frequently, or allow to stand overnight. Transfer the precipitate to a small filter or filtering crucible. Wash, and ignite as directed under II, 54. If $Mn_2P_2O_4$ is present, correct for it as directed under II, 54.

(8) With minor changes suggested by the referee, the volumetric method for the determination of acid-soluble manganese in fertilizers and manganese salts (*This Journal,* 21, 292) was adopted as tentative. The preparation of the standard ferrous sulfate solution should be changed to read as follows: "0.091 N 25.3 g of $FeSO_4 \cdot 7H_2O$ and 25 cc of H_2SO_4 in 1 liter of soln. Standardize with the 0.0910 N $KMnO_4$."

(9) The tentative method for the determination of acid- and base-forming quality of fertilizers (p. 34, 55) was modified as follows:

(a) The methyl red indicator was changed to the following:

Mixed indicator.—Weigh 0.1 g of bromocresol green and 0.02 g of methyl orange into an agate mortar, triturate, and slowly add about 2 cc of 0.1 N NaOH. Dilute to 100 cc with H_2O.

(b) The use of a filter paper cone for the prevention of spattering was made optional.

(c) Under "Determination," line 17, the direction "add 10 drops, etc." was changed to read "add 0.4 cc of the mixed indicator."

III. SEWAGE*

IV. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or other changes.

V. AGRICULTURAL DUST*

VI. INSECTICIDES AND FUNGICIDES

No additions, deletions, or other changes.

VII. CAUSTIC POISONS

No additions, deletions, or other changes.

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* Subjects for future study.

VIII. NAVAL STORES

No additions, deletions, or other changes.

IX. PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

No additions, deletions, or other changes.

X. LEATHERS

No additions, deletions, or other changes.

XI. TANNING MATERIALS

No additions, deletions, or other changes.

XII. PLANTS

(1) Volumetric method II for the determination of chlorine (p. 131, **38**) was modified and adopted as official (first action). The changes are included in the following statements:

(2) The explanatory note under the title of par. **38**, p. 131, was transposed to par. **34**, and placed under the title "Chlorine."

(3) The following explanatory note was placed under the heading "Preparation of Solution," par. **34**:

Complete retention of Cl in each kind of material should be verified by trial since losses can occur, especially with samples high in carbohydrates, (*This Journal*, **11**, 209(1928); **12**, 195(1929), if insufficient Na_2CO_3 is present during the ignition, or in any case if excessive temperatures are used, *Ibid.*, **21**, 107(1938).

(4) The following explanatory note was placed under the title of par. **36**:

(The limit of accuracy of this titration is considered to be approximately 10.2 mg of Cl, (*Am. Chem. Soc.*, **37**, 1128(1915), hence an accuracy of 1.0 would require samples containing not less than 20 mg.)

(5) The following revisions were made in the tentative method for the determination of chlorine in plants (p. 131, **38**) in order to make it conform to the text of the method as recommended for adoption as official (first action) at the 1938 Annual Meeting of the Association:

Page 131, **38(a)**, change "4.6826" to read "4.6822."

Page 132, **38(e)**, change to read, "Add 35 ml of H_2SO_4 to each liter of H_2O, boil 5–10 min., and cool to room temp."

Page 132, **38(f)**, delete par. (f) and insert instead the following: "*Iodine soln.*—Shake a large excess of I crystals in a glass-stoppered bottle nearly filled with Reagent(e). Decant, and discard the soln. Repeat the process but decant the soln into a glass-stoppered bottle. Test the soln by adding 25 ml of it to 25 ml of Reagent (e), followed by 5 ml of Reagent (d). (No blue color should appear after 5 min., and the color produced by a small amount of Reagent (a) should be discharged by an equivalent amount of Reagent (c). If the soln gives a blue color when tested, compute the amount of Reagent (c) needed to treat the remainder of the decanted soln from the excess of Reagent (c) over Reagent (a) observed in the test titrations. Add twice that amount and test as before."

Page 132, 39, line 9, delete the remainder of this sentence beginning with the words, "fades slowly, etc." and insert in their place the words, "begins to fade slowly."

Page 132, 39, line 18, change the word "ash" to "wash."

Page 132, 39, line 2 from bottom of page, revise this sentence to read: "When the digest is cool, add 175 ml of H_2O, boil 5–10 min., and cool to room temp."

XIII. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

No additions, deletions, or other changes.

XIV. MALT BEVERAGES, SIRUPS, AND EXTRACTS, AND BREWING MATERIALS

The following pressure air method for the determination of carbon dioxide in beer was adopted as tentative:

CARBON DIOXIDE IN BEER
Pressure Air Method

Disconnect the bottle or can and determine the head space volume as follows:

If the sample is a bottle, fill with H_2O to the top and pour off into a graduated cylinder to the scratch mark. The number of ml of H_2O thus poured off represents head space in ml.

If the sample is a can, weigh empty can after pouring out all remaining beer. The difference represents the weight of beer, which divided by the sp. gr. of the beer will give volume of beer in ml. Fill the empty can with H_2O and weigh. Weight of H_2O in g is also the volume in ml, so that the difference between volume of H_2O and volume of beer represents head space in ml.

Calculate CO_2 by weight by the following formula:

$$\% \ CO_2 = \left[P - \left(\frac{ml \ of \ air}{ml \ of \ head \ space} \times 14.7 \right) \right] \times 0.00965, \text{ in which}$$

P = absolute pressure in pounds per sq. in. at 25° = (ordinary gage pressure +14.7).

NOTES: Pounds per sq. in. ×0.070307 = kg per sq. cm. For routine work 15 may conveniently be substituted for 14.7.

XV. WINES

No additions, deletions, or other changes.

XVI. DISTILLED SPIRITS

The following sulfite method for the determination of aldehydes in whiskey and other potable spirits was adopted as tentative:

ALDEHYDES IN WHISKEY OR OTHER POTABLE SPIRITS
REAGENTS

(a) *0.05 N sodium thiosulfate soln.*—Standardize against a 0.05 *N* $K_2Cr_2O_7$ soln as follows: Place 20 cc of *0.05 N* $K_2Cr_2O_7$ soln in a glass-stoppered flask and add 5 cc of a 15% KI soln. Add 2.5 cc of HCl and dilute with 100 cc of CO_2-free H_2O, then titrate at once the liberated I with the thiosulfate soln until the yellow color has almost disappeared; add a few drops of starch indicator, and continue, with constant shaking, the addition of thiosulfate soln until the blue color just disappears.

(b) *0.05 N iodine soln.*—Standardize this soln against the thiosulfate soln.

(c) *Sodium bisulfite soln.*—Approximately 0.05 *N*. With each series of determinations, determine the strength of this soln in terms of the I soln. (This soln will not deteriorate nearly so fast when it contains 5–10% of alcohol.)

DETERMINATION

Run 50 cc of sample into an Erlenmeyer flask and add 10 cc of H_2O. Distil off 50 cc or slightly more, transfer the distillate to a glass-stoppered flask or bottle, and add about 150 cc of CO_2-free H_2O. Using a pipet, add 25 cc of the bisulfite soln and allow to stand for about 30 min., shaking occasionally. Add an excess (about 30 cc) of the standard I soln, titrate this excess with the thiosulfate soln, and calculate as acetaldehyde. 1 cc of 0.05 *N* soln = 0.0011 g of acetaldehyde.

NOTES: Do not add the starch indicator until the yellow color of the I soln has almost disappeared. As the end point is approached the soln will have a decided violet tint rather than a blue, as is customary with I and starch. If the end point is in doubt, add a little more of the starch indicator. The formation of a bluish-violet color indicates that the end point has not been reached. Always run a blank on the bisulfite soln along with each series of aldehyde determinations.

XVII. BAKING POWDERS AND BAKING CHEMICALS

The following methods for the determination of free tartaric acid, cream of tartar, and total tartaric acid were adopted as official (first action):

CREAM OF TARTAR AND FREE TARTARIC ACID
Total, Combined, and Free Tartaric Acid

To 2.5 g of the baking powder in a 250 cc volumetric flask, add 100 cc of H_2O at about 50°, and allow to stand at room temp. for about 30 min., shaking occasionally. Cool, dilute to mark with H_2O, shake vigorously, and filter through a large fluted paper. Pipet 2 portions of 100 cc each of the *clear* filtrate into 250 cc beakers and evaporate to about 20 cc. To one portion add 3.5 cc of approximately normal KOH. Mix well and add 2 cc of glacial acetic acid. Again mix well and add 100 cc of 95% alcohol, stirring constantly. Treat the other portion in a similar manner, but use normal NaOH instead of KOH. Cool the mixtures to about 15°, stir vigorously for about 1 min., and allow to remain in the refrigerator overnight. Collect the precipitate in a Gooch on a thin, tightly tamped pad of asbestos. Rinse the beaker with about 75 cc of ice-cold 80% alcohol, carefully washing down the sides of the beaker. Finally, wash the sides of the crucible with 25 cc of the alcohol and suck dry. Transfer the contents of the crucible to the original beaker with about 100 cc of hot H_2O and titrate with 0.1 *N* alkali, using phenolphthalein indicator. Designate the titer of the portion treated with KOH as "A" and that treated with NaOH as "B."

CALCULATIONS

$$\frac{2.5}{250} \, 100 = 1 \text{ g of powder in the aliquot.}$$

Total tartaric acid—
0.015 (A + 0.6) × 100 or *1.5 (A + 0.6)*
Combined tartaric acid (cream of tartar)—
0.0188 (B + 0.6) × 100 or *1.88 (B + 0.6)*.
Free tartaric acid—
0.015 (A − B) × 100 or *1.5 (A − B)*.
In the above formulas "0.6" represents the solubility of the cream of tartar in the reaction mixture in terms of 0.1 *N* alkali.

Free Tartaric Acid (Direct Determination)

REAGENT

Saturated alcohol.—To about 50 g of purest cream of tartar (finely powdered) in an Erlenmeyer flask, add about 100 cc of 95% alcohol and 100 cc of H_2O, shake vigorously for several minutes, and allow to stand 15 min., shaking occasionally. Filter on paper in a Büchner funnel, and wash the salt with about 200 cc of diluted 95% alcohol (1 +1), then with 95% alcohol, and finally with ether. Dry at the temp. of boiling H_2O. To 500 cc of *absolute* alcohol add about 5 g of the purified cream of tartar and allow to stand 2 hours, shaking occasionally. If the cream of tartar has been properly purified a blank (50 cc of $CHCl_3$+150 cc of the saturated alcohol) should not require more than 0.15 cc of 1 N alkali to neutralize 100 cc of the mixture.

DETERMINATION

Weigh 1.25 g of the baking powder into an absolutely *dry* 200 cc volumetric flask, add 50 cc of $CHCl_3$, and allow to stand about 5 min., shaking occasionally. (If, upon the addition of the $CHCl_3$, the powder sticks to the bottom of the flask, moisture is indicated and the determination should be discarded.) Add 100 cc of the saturated alcohol, shake for about 5 min., and allow to stand 30 min., shaking at frequent intervals. (It is not necessary to filter the alcohol reagent.) Make to mark with the saturated alcohol, shake a few minutes, and filter through a large fluted paper. Titrate 100 cc of the clear filtrate with 0.1 N alkali (phenolphthalein). The quantity (cc) of alkali (X) used $\times 1.2$ = the percentage of free tartartic acid.

$$\frac{1.25}{200} \times 100 = 0.625 \text{ g of powder in aliquot;}$$

$$\frac{X(0.0075 \times 100)}{0.625} = 1.2X.$$

XVIII. COFFEE AND TEA

XIX. CACAO PRODUCTS

No additions, deletions, or other changes.

XX. CEREAL FOODS

(1) The tentative method for preparation of sample of bread (p. 221, 50) was adopted as official (final action).

(2) The tentative method for collection and preparation of sample of macaroni products (p. 228, 68) was adopted as official (final action).

(3) In 38(c), p. 216, the statement, "0.0001 mg of N as nitrite," was changed to "0.0001 mg of N" (final action).

(4) The tentative method for the determination of acidity of water extract (p. 208, 13) was deleted.

(5) The following method for the determination of fat acidity of flour was adopted as tentative:

FAT ACIDITY OF FLOUR

Extract duplicate 10 g samples with petroleum ether for approximately 16 hours, using a Soxhlet (or similar extraction apparatus) and double thickness paper or Alundum R.A. 360 thimbles.

Completely remove the solvent from the extract by evaporation on the steam bath. Dissolve the extract in the extraction flask with 50 cc of a mixture of equal parts by volume of ethyl alcohol and benzene, and containing approximately 0.02% of phenolphthalein.

Titrate the dissolved extract with carbonate-free standard alkali to a distinct pink color. For convenience, use exactly 0.0178 N alkali for the titration in order to simplify calculations. 1 cc of this soln = 1 mg of K_2O_4.

Make a blank titration on 50 cc of the benzene-alcohol mixture and subtract the value obtained from the titration value of the sample.

Report fat acidity as the number of mg of K_2O_4 required to neutralize the free fatty acids from 100 g of flour on a dry-matter basis.

(6) As modified by the associate referee, the tentative method for the determination of extract soluble in cold water (p. 213, **30**) was adopted as official (first action). The modified method follows:

EXTRACT SOLUBLE IN COLD WATER

Weigh 20 g of the flour into a 500 cc Erlenmeyer flask and add gradually 200 cc of H_2O at a temp. approximating 0°. Shake vigorously when about 50 cc of H_2O has been added, and continue shaking during the addition of the remaining H_2O. Allow the mixture to stand at 0° for 40 min., shaking occasionally. Filter rapidly, returning the first runnings to the filter, until a clear filtrate is obtained. Pipet 20 cc of the clear filtrate into a weighed dish, evaporate to dryness on a steam bath, and dry to constant weight in a vacuum oven at about 100° for periods of 30 min.

(7) The method for the determination of apparent viscosity of flour (*This Journal*, **20**, 380) was adopted as official (final action).

XXI. COLORING MATTERS IN FOODS

No additions, deletions, or other changes.

XXII. DAIRY PRODUCTS

The following method for the estimation of mold mycelia in butter was adopted as tentative:

MOLD MYCELIA IN BUTTER[1]

REAGENT

Gum soln.—Make up 1 liter of a 0.75% soln of carob bean gum with 2% of added formaldehyde as a preservative. (The dry gum may be conveniently added by first mixing it in 10–15 cc of 95% alcohol and stirring this mixture rapidly into the H_2O.) Gently heat the soln to boiling to drive off alcohol and air, and sustain the heating for 25–30 min. Add formaldehyde on cooling. Use the clear supernatant soln, free from cells, left when the cellular elements in the gum gradually settle out. (A similar soln made with gum tragacanth may also be used for this purpose.)

PROCEDURE

Make a careful examination of the surface of the butter to insure freedom from surface mold growth and note any mold growth visible. In order to remove possibility of contamination of any surface mold not visible, scrape off and discard ⅛″ of the surface, after which take a sample from the exposed surface.

Weigh out 1 g of butter by means of a ¼ teaspoon measure. Measure out 7 cc of the hot gum soln and, with the spoon bottom-side-up over a 50 cc beaker, pour 2

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[1] *This Journal*, **20**, 93 (1937).

or 3 cc of the hot soln over the spoon. (This quantity is usually sufficient to loosen the butter and cause it to slide into the beaker.) Use the remainder of the 7 cc of soln to rinse the remaining fat from the spoon.

Stir the mixture until the soln is well mixed and fat globules are 0.1–0.2 mm in diameter. (The stirring necessary to obtain a uniform sample must be determined by experience.)

Mount a portion of the mixture on the mold-counting slide and estimate the mold as directed under **XXXV**, 27, 28. Report no field positive unless the combined length of the two longest filaments exceeds ⅛ of the diameter of the field.

XXIII. EGGS AND EGG PRODUCTS

(1) The official method for the determination of chlorine (p. 301, 16, 17) was changed to the following and adopted as official (first action):

CHLORINE

(a) *Liquid Eggs* (in absence of added salt).—From the well-mixed sample, 1(a) or (b), weigh accurately, by difference, into a 150 cc low-form Pyrex beaker, approximately 4 g of yolk, 7 g of whole eggs, or 10 g of whites; add 20 cc of 10% Na_2CO_3 soln, mix, and evaporate to dryness on an electric hot plate or overnight at 100°. Transfer the beaker while hot to an electric muffle heated to 500° (faint redness), and allow to remain at that temp. for 1 hour. Cool, add a few drops of H_2O, and break up the charge with a glass rod. Add 50 cc of H_2O, cover the beaker with a watchglass, add slowly 20 cc of HNO_3 (1+3), mix, filter, and wash the charred material and filter thoroughly with H_2O. Proceed as directed in one of the following alternatives:

(1) To the combined filtrate and washings add a known volume of 0.1 N $AgNO_3$ in slight excess and proceed as directed in **XII**, 37.

(2) Collect the filtrate and washings in a 250 cc flask, keeping the total volume of filtrate to 180 cc or less. Add a known volume of 0.1 N $AgNO_3$ in slight excess and make to volume. Filter, and determine Cl_2 in an aliquot as directed in **XII**, 37.

(b) *Liquid Eggs* (in presence of added salt).—from the well-mixed sample, 1(a) or (b), weigh 1–2 g accurately, by difference, into a 150 cc low-form Pyrex beaker, and proceed as directed under (a),

(c) *Dried Eggs.*—From the well-mixed sample, 1(c), transfer to a 150 cc low-form Pyrex beaker, 2 g of whole eggs or yolks, or 1 g of whites, and proceed as directed under (a).

(2) The method for the determination of dextrose and sucrose (p. 301, 18, 19), official (first action), was modified as follows:

(a) The words "add 5 cc of HCl, and allow to stand overnight," in line 4 of par. 19, were changed to read, "and invert the sucrose as directed under **XXXIV**, 23(b) or (c)."

(b) The following paragraph was added at the end of 18(a):

To correct for the error due to the volume occupied by the precipitate in samples containing added sucrose, repeat the determination, weighing the same amount of sample into a 500 cc volumetric flask containing 1 g of $CaCO_3$ and 100 cc of 5% salt soln. Add, with continuous mixing, 260 cc of 95% alcohol. Allow to stand a few minutes for gas bubbles to rise to the surface, cool to room temp., fill to the mark with H_2O, shake, and filter through a 18.5 cm folded filter. Transfer 300 cc of filtrate to a 400 cc beaker, evaporate to 20–30 cc, and proceed as directed in **18**. To obtain the amount of sucrose subtract the percentage of sucrose obtained in the

250 cc dilution determination from twice the percentage obtained in the 500 cc dilution determination.

XXIV. FISH AND OTHER MARINE PRODUCTS

No additions, deletions, or other changes.

XXV. FLAVORING EXTRACTS

No additions, deletions, or other changes.

XXVI. FRUITS AND FRUIT PRODUCTS

(1) The following changes in the methods for the analysis of preserves and jams were adopted as official (first action):

(a) *Preparation of Sample—Official* (p. 319, 2(c)).—Insert the following after the word "grinding," line 5: "Set the burrs or the blades of the food chopper as closely as possible without crushing the seeds. If the container is a No. 10 can or smaller, grind the entire contents. Mix well the contents of larger containers by stirring, and remove a portion for grinding."

(b) *Water-Insoluble Solids—Tentative* (p. 320, 7).—Delete the entire paragraph following the first sentence, and substitute the following: "As the filtering medium, use a weighed piece of cotton 5″ square, of a thickness about one-half that of the layer in the ordinary 16-ounce roll of absorbent cotton. Tear a piece of the cotton off one corner and use to plug the neck of the funnel lightly. Then arrange the large piece in the funnel, and filter the sample. Pour the hot distilled H_2O in such a way that the pulp is loosened from the cotton with each addition (usually 700–800 cc of filtrate is collected). Fold the cotton and contents and remove the excess H_2O by gently squeezing the cotton while it is still in the funnel. Dry the material to constant weight at 100°″".

(c) *Total Ash—Official* (p. 321, 9).—Add the following sentences to the directions: "In case of excessive swelling or foaming, add 2–3 drops of ashless olive oil, as provided in **XXXIV, 8** and **9**. Moisten the partially ashed residue and after drying on the steam bath and hot-plate ash in the muffle."

(d) *Alcohol Precipitate—Tentative* (p. 324, 21).—Insert the following sentence in line 6: "Do not permit the alcohol precipitate to dry before transferring it from the paper."

(2) The official method for the determination of phosphoric acid (P_2O_5) in wines (**XV**, p. 166, 19) was adopted as tentative for the same determination in fruits and fruit products. The method will read as follows:

PHOSPHORIC ACID—OFFICIAL (FIRST ACTION)

Dissolve the ash, 9, in 50 cc of boiling HNO_3 (1 +9), filter, wash the paper, and determine P_2O_5 in the combined filtrate and washings as directed under **II, 9** or **12**.

XXVII. GRAIN AND STOCK FEEDS

(1) The following method for the determination of manganese in grain and stock feeds was adopted as tentative:

MANGANESE IN GRAIN AND STOCK FEEDS[1]

Ash a 5 g sample at dull-red heat in a porcelain evaporating dish. When cool, add 2 cc of H_2SO_4 and 5 cc of HNO_3. Evaporate to white fumes. If carbon is not

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[1] Willard and Greathouse, *J. Am. Chem. Soc.*, **39**, 2366 (1917): G. Frederick Smith Chemical Co. Publications, 1, 2nd ed., August (1933).

completely destroyed, add further portions of HNO₃, boiling after each addition. Cool slightly and add 25 cc of H₂O in which 1 cc of 85% H₃PO₄ has been dissolved. Cool, and let stand to allow precipitation of CaSO₄ and other insoluble matter. Filter thru a mat of acid-washed asbestos on a Gooch crucible and wash with H₂O. Evaporate to less than 50 cc and add approximately 0.3 g of KIO₄. Mix, and heat below the boiling point for 30 min., or until maximum color development. Cool, and dilute to an accurately measured volume, usually 50 or 100 cc, with H₂SO₄ (5+95). The final soln should contain not more than 2 mg of Mn and 5–15 cc of H₂SO₄ plus H₃PO₄ in 100 cc. Compare with a standard KMnO₄ soln in a colorimeter. Calculate p.p.m. of Mn in the sample.

Standard potassium permanganate.—Dissolve 1.4385 g of C.P. KMnO₄ by boiling with H₂SO₄ (5+95). Dilute to 1 liter in a volumetric flask. Standardize by titration with 0.1000 g of oven-dry Na₂C₂O₄, dissolved in 100 cc of H₂SO₄ (5+95), keeping the temp. above 60°. (The soln should contain 500 p.p.m. of Mn. Add 0.3 g of KIO₄. Protect from light. For a working standard dilute this soln with H₂SO₄ (5+95) to a known concentration approximately like that to be compared.

(2) The Peterson-Hughes method for the determination of carotene, and specifying the use of the spectrophotometer or the 0.1 per cent potassium dichromate standard (*This Journal*, **20**, 464) was adopted as tentative. The method follows:

CAROTENE

EXTRACTION OF CAROTENE

Weigh out the samples (1–5 g), transfer to a 200 cc Erlenmeyer flask, and to each gram of sample add 20 cc of a freshly prepared, saturated soln of KOH in ethyl alcohol. Fit the flasks with reflux condensers, and boil the contents on a steam bath or hot plate for 30 min. If portions of the sample collect on the sides of the flask, wash down with alcohol from a wash bottle. Cool the contents of the flask. (The volume of petroleum ether may be reduced by direct filtration, after cooling, thru a sintered glass funnel of No. 3 porosity. The residue should be extracted with a small portion of petroleum ether until the solvent is colorless. Proceed as outlined in the method.) Add 100 cc of Skellysolve (b. p. 60–70°), or petroleum ether, and after shaking for a minute or so and allowing the sediment to settle, decant the Skellysolve-alcohol mixture into a 500 cc separatory funnel. Repeat this procedure twice more with 25 cc portions of Skellysolve, breaking up the residue, which sometimes forms an adherent mass, by shaking with 10–15 cc of 95% alcohol. After two or three additional extractions with 20 cc portions of Skellysolve (the soln usually comes off colorless) discard the residue.

Pour gently about 100 cc of H₂O thru the alcohol Skellysolve soln in the separatory funnel. Draw off the alkaline alcohol-H₂O soln from the bottom of the funnel, and re-extract three times by shaking gently with 30 cc portions of Skellysolve, using two other separatory funnels. Combine the Skellysolve extracts and wash them with 50 cc portions of H₂O until free from alkali, as indicated by the absence of color in the wash H₂O when treated with phenolphthalein (about 10 washings). (One washing with H₂O will usually suffice since all the alkali is removed in subsequent extractions with methyl alcohol. If alkali removal is desired, the use of larger amounts of H₂O (about 100 cc) will reduce the number of washings.) Any small amount of alkali remaining will be removed by subsequent methyl alcohol and H₂O washings.

Remove xanthophyll from the Skellysolve soln by extraction with 25 cc portions of 90% methyl alcohol (90 cc CH₃OH +10 cc H₂O), shaking for 2 minutes. Continue these extractions until the wash alcohol comes off colorless. (This may

Extinction coefficients

WAVE LENGTH, Å	SKELLYSOLVE B.P. 60-70°	PETROLEUM ETHER B.P. 40-60°
4500	238	243
4550		231
4700	200	207
4800	212	212

require 6–12 washings, depending on the amount of xanthophyll in the sample.) Wash the Skellysolve soln containing the carotene twice with 50 cc of H_2O to remove the alcohol, and adjust to volume (either dilution or concentration under reduced pressure) to obtain convenient concentration for measurement of the carotene. Filter into a volumetric flask thru filter paper upon which is placed a small amount of anhydrous Na_2SO_4. After making the carotene soln up to definite volume, determine the concentration by the spectrophotometer, photoelectric colorimeter, or colorimeter by comparison with 0.1% or 0.036% $K_2Cr_2O_7$.

Table for calculating carotene

0.1% $K_2Cr_2O_7$	CAROTENE	0.1% $K_2Cr_2O_7$	CAROTENE
mm	p.p.m.	mm	p.p.m.
1.0	0.5	6.6	4.1
1.2	0.7	6.8	4.2
1.4	0.8	7.0	4.3
1.6	0.9	7.2	4.5
1.8	1.0	7.4	4.6
2.0	1.2	7.6	4.7
2.2	1.4	7.8	4.8
2.4	1.5	8.0	4.9
2.6	1.6	8.2	5.0
2.8	1.7	8.4	5.2
3.0	1.8	8.6	5.3
3.2	2.0	8.8	5.4
3.4	2.1	9.0	5.6
3.6	2.2	9.2	5.8
3.8	2.3	9.4	5.9
4.0	2.5	9.6	6.0
4.2	2.6	9.8	6.1
4.4	2.7	10.0	6.3
4.6	2.8	10.2	6.5
4.8	2.9	10.4	6.7
5.0	3.1	10.6	6.8
5.2	3.2	10.8	6.9
5.4	3.4	11.0	7.1
5.6	3.5	11.2	7.3
5.8	3.6	11.4	7.4
6.0	3.8	11.6	7.5
6.2	3.9	11.8	7.6
6.4	4.0	12.0	7.8

DETERMINATION

For each determination by the spectrophotometric method make optical density measurements at wave lengths of 4500, 4700, and 4800 Å.U. Using the absorption coefficients calculated for beta carotene at these wave lengths, determine the carotene concentration for each wave length, take the average and report results to 0.1 p.p.m.

Or, estimate the amount of carotene in the sample by comparing it colorimetrically against 0.1% $K_2Cr_2O_7$. Put the soln of the sample in the left-hand cup of the colorimeter and set the scale at 0.5 cm, 1 cm, 2 cm, 3 cm, or 4 cm, according to the amount of color present. Vary the depth of the dichromate soln in the right-hand cup until the density of color in both cups is equal, and make eight independent readings, recording them in mm. Average the readings. Make the dichromate readings between 4 mm and 12 mm on the colorimeter. If necessary, make a reading below 4 mm, but repeat the analysis with a larger sample.

By use of the table transform the depth in mm of 0.1% dichromate into p.p.m. of carotene. Then calculate the p.p.m. of carotene actually in the sample by use of the following formula:

$$p = \frac{\text{p.p.m. of carotene (from table)} \times \text{cc of soln}}{\text{g of sample} \times \text{cm depth of sample soln}}.$$

Report carotene of 0.1 p.p.m.

(3) The tentative method for vitamin D assay by preventive biological test (p. 351, 55) was revised as follows:

VITAMIN D ASSAY BY PREVENTIVE BIOLOGICAL TEST

(Applicable to fish and fish liver oils and their extracts, and to materials used for supplementing the vitamin D content of feeds. Not applicable to irradiated ergosterol products or to irradiated yeast unless recommended for poultry.)

This essay is a comparison under conditions specified below of the efficacy of the product under assay with the U.S.P. Reference Cod Liver Oil in controlling the ash content of the bones of growing chicks.

The basal ration is a uniform mixture in the proportions designated, of the following ingredients which have been finely ground:

BASIC RACHITIC RATION	*per cent*
Ground yellow corn	58
Wheat flour middlings or Wheat Gray shorts	25
Crude domestic acid precipitated casein	12
Calcium phosphate (precipitated)	2
Iodized salt (0.02% KI)	1
Non-irradiated yeast (7% minimum N)	2

To each kg of the above mixture add 0.2 g of $MnSO_4 \cdot 4H_2O$.

PROCEDURE

Conduct the assay on groups of chicks kept in cages provided with screen bottoms and away from sunshine or other source of actinic light that may influence calcification. Keep the cages in rooms in which wide variations in temp. are prevented (constant temp. preferred). Unless the temp. of the room is adequately controlled provide each cage with a suitable electrical heating device. Start all the birds to be used in one assay on the same day and keep all conditions of environment for all the groups in the assay uniform.

Perform the assay on groups of one- or two-day-old white Leghorn chicks as specified below. Provide for one or more negative control groups that receive no vitamin D, one or more positive control groups that receive the U.S.P. Reference Cod Liver Oil, and one or more assay groups for each product to be assayed. Have the positive control and assay groups consist of not less than twenty birds, and the negative control group consist of not less than ten birds. Make up the rations for all the groups in the assay from one batch of the basal ration. Add the Reference Cod Liver Oil to the basal ration in such quantities as to produce a measurable increase in percentage of bone ash above that obtained in the negative control group (it is not possible to make comparisons if maximum bone ash is obtained). Add the assay product to the basal ration in such quantities as to permit a direct comparison in the response of assay and positive control groups. To the basal ration of the negative control group add corn oil equal in quantity to the maximum quantity of oil fed to any group in the assay and add corn oil to the rations of the other groups until the total quantity of corn oil and oil containing vitamin D is equal to the quantity of corn oil added to the ration of the negative control group. Feed the chicks in the respective groups the prescribed ration and water (U.S.P. or distilled water) *ad libitum* for 21 days. Discard all chicks that weigh 100 g or less and all chicks that show abnormality or disease not related to vitamin D deficiency. At least fifteen chicks must remain in each reference or assay group that is used in calculating the vitamin D potency of an assay product.

Kill the chicks; remove the left tibia of each bird and clean of adhering tissue. (To facilitate removal of adhering tissue the bones may be placed in boiling H_2O for not more than 2 min. The bones may be preserved in alcohol for extraction.) Completely extract the bones with a suitable fat solvent or solvents (20 hours with hot 95% ethyl alcohol, followed by 20 hours with ethyl ether may be used, and the bones may be crushed to facilitate extraction.) Dry the extracted bones to constant weight in a moisture oven, cool in a desiccator, and weigh. Ash the moisture and fat-free bones from each group of birds in a muffle furnace to constant weight at any given temp. between 450 and 550°, or if preferred for 1 hour at approximately 850°. (The ash determination may be made on individual bones if desired.) Cool the ash in a desiccator and weigh. Use the specific procedure adopted for extraction, drying and ashing of the bones consistently throughout any one assay.

INTERPRETATION OF RESULTS

One A.O.A.C. chick unit of vitamin D is equal in biological activity for the chick to one unit of vitamin D in the U.S.P. Reference Cod Liver Oil in this method of assay. The product under assay meets its declared vitamin potency in A.O.A.C. chick units of vitamin D if the percentage of ash in the moisture and fat-free bone produced in the assay groups by a given number of units of vitamin D is equal to or greater than the percentage of ash produced by the same number of units of vitamin D from the U.S.P. Reference Cod Liver Oil.

XXVIII. MEAT AND MEAT PRODUCTS

(1) In place of the phenoldisulfonic acid method for the determination of nitrates in meat products (p. 356, 14, 15) the following m-xylenol method was adopted as tentative:

Xylenol Method

APPARATUS

Use a simple distillation apparatus, including a distillation bulb. A glass condenser of a type utilizing a thin, rapidly moving film of H_2O as a cooling medium

(West type) is recommended. Quickly remove any nitro-xylenol solidifying in the condenser by stopping the flow of H_2O and allowing the condenser to become warm.

REAGENTS

(a) *Nitro-xylenol.*—1-hydroxy, 2, 4-dimethylbenzene. Eastman's preparation No. 1150, or equivalent.

(b) *Silver ammonium hydroxide.*—Dissolve 5 g of *nitrate-free* Ag_2SO_4 in 60 cc of NH_4OH. Heat the mixture to boiling, concentrate to about 30 cc, cool, and dilute to 100 cc with H_2O.

(c) *Bromocresol green indicator.*—Dissolve 0.1 g of bromocresol green in 1.5 cc of 0.1 N NaOH, and make up to 100 cc with H_2O.

(d) *Standard nitrate soln.*—Dissolve 0.1804 g of recrystallized KNO_3 in H_2O and make up to 1 liter, or dilute 17.85 cc of HNO_3 to 1 liter. 10 cc contains 0.25 mg of nitrate nitrogen.

DETERMINATION

Mix 5–10 g of the finely comminuted and thoroly mixed sample with 80 cc of warm H_2O. Break up all lumps and heat on the steam bath for 1 hour with occasional stirring. Transfer to a 100 cc volumetric flask, cool, make up to mark, and mix. Filter, or allow to settle, and pipet 40 cc of the filtrate, or supernatant liquid, into a 50 cc volumetric flask. (No correction for the volume occupied by the meat is necessary.) Add 3 drops of the bromocresol green indicator. Add H_2SO_4 (1+10) dropwise until the color changes to yellow. Oxidize nitrites to nitrates by adding 0.2 N $KMnO_4$ soln dropwise with shaking until a faint pink color remains for approximately 1 min. Add 1 cc of H_2SO_4 (1+10). Add 1 cc of phosphotungstic acid soln (20 g in 100 cc). Make up to mark, mix, and filter.

Measure into a 500 cc flask (an Erlenmeyer is satisfactory) an aliquot (not more than 20 cc) containing from 0.025 to 0.25 mg of nitrate nitrogen. (If more than 20 cc is required, make slightly alkaline and concentrate by evaporation.) Add a sufficient quantity of the silver NH_4OH soln to precipitate all chlorides and most of the excess phosphotungstic acid. (A slight excess of the silver reagent is not harmful; 1 or 2 cc is usually sufficient.) Without decanting or filtering, add a volume of H_2SO_4 (3+1) approximately three times the volume of liquid in the flask. Stopper the flask, mix, cool to about 35° C., add 0.05 cc (1–2 drops) of the m-xylenol, stopper, shake, and hold at 30–40° for 30 min.

(A yellow to brownish yellow color, indicative of nitrates, will appear. A bright red precipitate, due to incomplete removal of phosphotungstic acid, may also appear. A slight excess of phosphotungstic acid causes no interference but a large excess may do so.)

After nitration is complete, add 150 cc of H_2O, taking care to wash off the stopper, and distil until 40–50 cc has passed over into a receiver containing 5 cc of NaOH (10 g per liter). Transfer the distillate to a 100 cc volumetric flask, make up to volume with H_2O, and determine nitrate nitrogen by comparing the color of a suitable aliquot with a set of graded color standards containing 0.003–0.006 mg of nitrate nitrogen.

Prepare the color standard from 10 cc of the nitrate standard as directed previously, using 0.05 cc of the m-xylenol and 30 cc of H_2SO_4 (3+1), and making up the distillate to 500 cc. Prepare the color standard fresh each day, as it becomes cloudy on standing.

(2) In the tentative method for the determination of coagulable nitrogen (p. 360, 28) the following change in the manner of using the indicator was adopted: In par. **28**, line 2, following the words, "Neutralize to

phenolphthalein," the following words were added: "using the indicator outside the soln to avoid subsequent interference in the determination of creatin, 31."

(3) The present tentative methods for the determination of copper and zinc in gelatin (p. 368, **64, 65**) were deleted.

(4) The following method for the determination of copper in gelatin was adopted as tentative:

COPPER

PREPARATION OF SAMPLE

Ash 20–40 g, preferably in a muffle, as directed under **XXVII, 8**, keeping the temp. low to avoid loss.

REAGENT

Standard copper soln.—0.3927 g of recrystallized $CuSO_4$ per liter. 1 cc = 0.1 mg of copper.

DETERMINATION

Moisten the ash with a small quantity of H_2O, add approximately 5 cc of HCl, and evaporate to dryness. Add 8 cc of HCl (1 + 1), heat to boiling, and transfer to a 50 cc Erlenmeyer flask, using enough wash H_2O to make the volume approximately 40 cc. Heat nearly to boiling, saturate with H_2S, stopper tightly, and allow to stand in a warm place for 30 min. or more. Filter into a 150 cc Erlenmeyer flask and wash promptly and thoroly with warm 1 : 20 HCl saturated with H_2S. Transfer the paper and precipitate to a 50 cc porcelain crucible and ignite in a muffle furnace at a temp. not exceeding that at which the gelatin was ashed. After ignition, cool, moisten ash with 1–2 cc of HNO_3, and evaporate to dryness on steam bath. Dissolve the residue in 1 cc of NH_4 acetate soln (500 g per liter). Filter into a 50 cc graduated flask, wash out crucible with warm H_2O, make up to mark, and mix. Measure out 25 cc into a 50 cc Nessler tube, add 5 cc of NH_4NO_3 soln, and make up to 50 cc. Add 0.2 cc of $K_4Fe(CN)_6 \cdot 3H_2O$ soln and mix. Match the color against tubes prepared in the same way from the standard Cu soln. Make up standards containing 2, 3, 4, 5, and 6 cc of the standard soln equivalent to 20, 30, 40, 50, and 60 p.p.m. of Cu if a 20 g sample is used and one-half of soln taken. Solutions giving a stronger reaction than 6 cc of the standard cannot be accurately compared. If a reaction stronger than that given by 6 cc of the standard is obtained, take an aliquot smaller than 25 cc and repeat the determination.

(5) The following method for the determination of zinc in gelatin was adopted as tentative:

ZINC[1]

Boil the filtrate and washings from the H_2S precipitate of Cu until all H_2S is removed. Add 1 cc of HNO_3 and continue the boiling until the volume is reduced to approximately 25 cc. Add 10 cc HN_4Cl (200 g per liter), make definitely alkaline with NH_4 hydrate, heat nearly to boiling, and filter into a 100 cc Erlenmeyer flask. Wash with warm alkaline NH_4Cl soln containing 50 g of NH_4Cl and 25 cc of NH_4 hydrate (sp. gr. 0.90) per liter. Neutralize the filtrate and washings with acetic acid, add 0.5 g of Na acetate and sufficient glacial acetic acid to make an excess of 2 cc for each 50 cc of soln. Warm the mixture on the steam bath and saturate with H_2S. Allow to stand in a warm place for approximately 30 min. Filter thru a small paper and wash thoroly with warm 1 : 100 acetic acid (1 + 1) saturated with H_2S. If the filtrate is turbid, return to flask, add a few drops of saturated $HgCl_2$ soln, shake, and

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[1] *Ind. Eng. Chem.*, **15**, 942 (1923).

filter again. Ignite in a tared Pt crucible at a dull red heat until completely ashed, then a few minutes at bright red heat. Weigh as ZnO. Weight of ZnO ×40,000 = p.p.m. of Zn if a 20 g sample was taken.

XXIX. METALS IN FOODS

(1) The colorimetric dithizone method for the determination of Pb on apples and pears (p. 391, **30**), including the following minor changes, was adopted as official (final action): Line 7, include in the parentheses the following statement: "If for the purpose of analysis the inclusion of the Pb content of stems and sepals is not desired, these may be discarded"; line 9, change "(1+49)" to "(2+99)"; line 10, delete "HNO_3 if the Pb is to be determined electrolytically, **33**)"; 6th line from end insert "exactly" after word "place," and delete "or HCl to conform to the kind of acid used in rinsing."

(2) The electrolytic determination of Pb in apple filtrate (p. 393, **33**) was changed to read as follows: "Transfer 200 cc of the acid filtrate to a separatory funnel, add the equivalent of 5 g of citric acid **(13(d))**, make ammoniacal, add 5 cc of the 10% KCN soln, extract with dithizone as directed in **16(a)**, and finally determine the Pb electrolytically as directed in **19** and **20**.

(3) The following method for the determination of selenium was adopted as tentative:

SELENIUM

PREPARATION OF SAMPLE

Place 5–10 g (dry weight) of the sample in a 600 cc Pyrex beaker or a Kjeldahl flask, add 0.5 g of HgO and a cooled mixture of 50 cc of H_2SO_4 and a volume of HNO_3 equal to 10 cc per g of sample taken. Mix thoroly and allow to stand 30 min. Heat *gently* until NO_2 fumes are no longer evolved and the soln turns to a dark brown or SO_2 fumes appear. Cool, and distil with $HBr+Br_2$. (The Hg can best be added in soln in HNO_3.)

ISOLATION

Add 25 cc of H_2O to the cold H_2SO_4 digest, cool again, and add 50–60 cc of HBr containing 0.5% by volume of free Br_2. Attach the flask to an all-glass distilling apparatus equipped with a thermometer and distil to 130°, keeping the receiving flask cool. If the distillate contains insoluble material, filter thru asbestos and wash. Saturate the filtrate with SO_2 gas, add 0.1 g of NH_2OH-HCl and warm on a steam bath to 80° for 15 min. Allow the mixture to cool and filter thru an asbestos Gooch or Jena glass filter No. 4 and wash. (The filtrate may be saved for recovery of HBr.)

VOLUMETRIC DETERMINATION WITH STARCH INDICATOR

Estimate the amount of the precipitate Se on the filter. (This estimate is used in determining the quantity of $Na_2S_2O_3$ soln to be added later in the titration.) Dissolve the Se in 1–2 cc of 48% HBr containing 1% by volume of Br_2, using a few drops to rinse the precipitation flask. Wash with a minimum quantity of H_2O so as to keep the volume of filtrate below 20 cc at most and at about 10 cc for amounts of Se of 20 gamma and under. Transfer the filtrate and washings to a 30 or 50 cc beaker. Prepare a few standards containing amounts of Se in the general range of the samples, and 2–3 blanks. Dilute standards and blanks to about the volume of

the samples and add the same volume of $HBr + Br_2$ as in the samples. To samples, standards, and blanks, add a strong soln of H_2SO_4 until the Br_2 color nearly disappears. (In case all the Br is reduced, add $HBr + Br_2$, dropwise until the color reappears.) Decolorize with 1–2 drops of 5% aqueous phenol. (It is desirable to reduce the color to a light yellow since tribromophenol is precipitated with excess bromine. The presence of the precipitate, while undesirable, does not ruin the determination.)

Use a stirrer and a 10 cc buret provided with an extension to dip into the soln being titrated, with the tip so constricted as to make possible the addition of the soln in 0.01–0.02 cc portions. Place the soln being titrated on a white surface with a white background and view by reflected light.

To the decolorized soln in the 30–50 cc beaker add about 1 cc of freshly prepared starch soln. Then add rapidly from a buret a moderate excess of 0.01, 0.001, or 0.0005 N $Na_2S_2O_3$, using the estimate of the precipitated Se as a guide. (1 cc of 0.001 N $Na_2S_2O_3$ is roughly equivalent to 200 gamma of Se; 1 cc of 0.001 N to 20 gamma; 1 cc of 0.0005 N to 10 gamma.)

Add about 2 cc more than the estimated equivalent of $Na_2S_2O_3$ and so select the normality as to keep the volume added between 2 and 10 cc. After about 20–30 seconds add rapidly from a buret a soln of I_2 (approximately the same strength as the $Na_2S_2O_3$ used) until a permanent blue color appears. If less than 1 cc of I_2 has been added, add 2 cc more of $Na_2S_2O_3$ and then I_2 until at least 1 cc is needed to give the blue color. Then add slowly from the dipping buret $Na_2S_2O_3$ of the same strength as before until the color is the same as a blank containing H_2O and 1 cc of starch soln.

CALCULATIONS

Add up the total volumes of I_2 and $Na_2S_2O_3$ for each determination.

Blanks.—Divide the volume of $Na_2S_2O_3$ by the volume of I_2 to get the factor for conversion of the volumes of I_2 to the equivalent volumes of $Na_2S_2O_3$. Average the results.

Standards.—Multiply the volumes of I_2 by the I_2-$Na_2S_2O_3$ conversion factor, and subtract the product from the total volume of $Na_2S_2O_3$. Divide this number into the quantity of Se in the standard to get the gamma Se/cc $Na_2S_2O_3$. Average the results.

Samples.—Calculate the volume of $Na_2S_2O_3$ used in the reduction of the Se as directed under "Standards" and multiply by the gamma Se/cc $Na_2S_2O_3$ value to get the total quantity of Se in the sample in gammas. Divide the total quantity by the weight of the sample in grams taken to get the p.p.m. of Se.

XXX. NUTS AND NUT PRODUCTS

No additions, deletions, or other changes.

XXXI. OILS, FATS, AND WAXES

(1) The official method for the determination of free fatty acids (p. 417, 30) was deleted (final action).

(2) The N.C.P.A. methods for the determination of free fatty acids in crude and in refined oils (*This Journal*, 21, 88) were adopted as official (final action).

(3) The refractometric method for the determination of oil in flaxseed (*This Journal*, 20, 74) was adopted as official (final action).

(4) The specifications for the titer thermometer (p. 408, 15) were amended to conform with the revised Bureau of Standards specifications as follows: Under *Range and subdivision*, change "62°" to "66°"; under

Total length, change "350–360 mm" to "370–380 mm"; under *Distance* change "62°'" to "66°'"; under *Filling above mercury,* add at end of line "or vacuum"; under *Graduation,* last line, change "every 2° mark" to "each multiple of 2°'"; under *Case,* change "62° C" to "66° C." These changes supersede all changes published in *This Journal,* since the 1935 revision of *Methods of Analysis.*

XXXII. PRESERVATIVES AND ARTIFICIAL SWEETENERS

No additions, deletions, or other changes.

XXXIII. SPICES AND OTHER CONDIMENTS

(1) The tentative method for the determination of volatile oil in spices (p. 447, 16) was made official for the same determination in marjoram and sage (first action).

(2) The method for the determination of ash in vinegar adopted as official (first action) last year (*This Journal,* 21, 89) was adopted as official (final action).

XXXIV. SUGARS AND SUGAR PRODUCTS

(1) The official method (Wein) for the determination of maltose (p. 484, 54, 55) was deleted (final action).

(2) The vacuum drying method of the International Commission for Uniform Methods of Sugar Analysis (*This Journal,* 21, 89) was adopted as official (final action).

(3) The International Scale of Refractive Indices of Sucrose Solutions at 20° C., 1936, International Sugar Journal Supplement, 39, 1–40 (1937), was adopted as official (final action).

(4) The International Temperature Correction Table, 1936, *ibid.,* was adopted as official (final action).

XXXV. VEGETABLES AND VEGETABLE PRODUCTS

(1) The method for the determination of alcohol-insoluble solids in canned peas published last year (*This Journal,* 21, 89) and adopted as official (first action) was adopted as official (final action) with the following minor clarifying modifications:

(a) Change the title to read, "Alcohol-Insoluble Solids in Canned Peas and Canned Dried Peas."

(b) Change the 3rd sentence on p. 90 to read as follows: "Grind the drained peas in a food chopper until the cotyledons are reduced to a smooth homogeneous paste, stir, and weigh 20 g of the ground material into a 600 cc beaker."

(c) Combine the first and second complete paragraphs on p. 90 to read as follows: "Fit into a Büchner funnel a filter paper of appropriate size, previously prepared by drying in a flat-bottomed dish for 2 hours at the temp. of boiling H_2O, covering with a tight-fitting cover, cooling in a desiccator, and weighing at once. Apply suction, and transfer the contents of the beaker to the Büchner funnel in such a manner as not to run over the edge of the paper. Suck dry and wash the material on the filter with 80% alcohol until the washings are clear and colorless."

(2) The method for the determination of chlorides in tomato juice published in 1937 (*This Journal*, 20, 78) and adopted as official (first action) in 1937 (*This Journal*, 21, 90) was adopted as official (final action) with the following minor clarifying changes: Under Determination, 6th line, change the sentence, "Cool, wash down, etc." to the following: "Cool, wash down the funnel and neck of the flask with H_2O, and add 1 cc of nitrobenzene and 1 cc of ferric indicator."

(3) The official method for preparation of sample (p. 497, 2) was extended to include canned fruit and was clarified by requiring tilting of the sieve during drainage and turning of all pieces to permit draining of cups or cavities.

XXXVI. VITAMINS

No additions, deletions, or other changes.

XXXVII. WATERS, BRINE AND SALT

The statement in 14(c), p. 506, "0.0001 mg of N as NO_2" was changed to read "0.0001 mg of N" (final action).

XXXVIII. RADIOACTIVITY

No additions, deletions, or other changes.

XXXIX. DRUGS

(1) The following microchemical tests for the identification of berberine, cotarnine, narceine and narcotine were adopted as tentative:

BERBERINE, COTARNINE, NARCEINE, AND NARCOTINE

REAGENTS

(a) *Platinic chloride soln.*—Dissolve 5 g of $H_2PtCl_6 \cdot 6H_2O$ in 100 cc of H_2O.

(b) *Mercuric chloride soln.*—Dissolve 5 g of $HgCl_2$ in 100 cc of H_2O.

(c) *Wagner's soln.*—Dissolve 1.25 g of I and 2 g of KI in 5 cc of H_2O and dilute to 100 cc.

(d) *Potassium ferrocyanide soln.*—*Freshly prepared.* Dissolve 5 g of $K_4Fe(CN)_6 \cdot 3H_2O$ in 100 cc of H_2O.

(e) *Potassium hydroxide soln.*—Dissolve 5 g of KOH in 100 cc of H_2O.

(f) *Hydrochloric acid.*—5%.

(g) *Ammonium hydroxide.*—10%.

(h) *Zinc potassium iodide soln.*—Dissolve 5 g of Zn acetate and 20 g of KI in 100 cc of H_2O.

IDENTIFICATION

Place a drop of the alkaloidal soln on a clean glass slide, add a drop of reagent by means of a clean glass rod, and without stirring or covering examine under the microscope, using low power (a magnification of 100–150 is suitable). Note the kind of crystals formed and compare their characteristics with a control specimen of the alkaloid in the same dilution.

Characteristics of microchemical tests for alkaloids

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Berberine	Hydrochloric acid	Saturated soln., fine yellow needles (Avoid excess reagent)
Cotarnine	Platinic chloride	1:200 hair-like crystals, yellow and curving
	Mercuric chloride	Colorless, long, branching needles
	Potassium ferrocyanide	Acidified with 1 drop of 5% HCl; globules that develop into dense, burr-shape crystals; also amber-brown plates
Narceine	Wagner's or zinc potassium iodide	1:400 blue, radiating needles, sometimes with yellow dichroism
	Platinic chloride	Beautiful, feathery rosettes develop in all solns
Narcotine	Potassium hydroxide or ammonium hydroxide	1:200 white amorphous precipitate, which crystallizes slowly; dense rosettes of needles

(2) The status of the microchemical methods for the identification of the alkaloids named below was advanced from tentative to official (first action):

Aconitine	Ephedrine	Quinidine
Apomorphine	Ethylmorphine	Quinine
Arecoline	Ethylhydrocupreine	Scopolamine
Benzylmorphine	Homatropine	Sparteine
Brucine	Hydrastine	Strychnine
Caffeine	Hydrastinine	Theobromine
Cinchonidine	Hyoscyamine	Theophylline
Cinchonine	Nicotine	Yohimbine
Cocaine	Papaverine	
Codeine	Procaine hydrochloride	

(3) The following microchemical tests for the identification of diallylbarbituric acid, mandelic acid, and sulfanilamide were adopted as tentative:

DIALLYLBARBITURIC ACID, MANDELIC ACID, AND SULFANILAMIDE

REAGENTS

(a) *Sodium nitrite.*—10%. Dissolve 10 g of $NaNO_2$ in H_2O to make 100 cc.

(b) *Benzaldehyde.*—N.F. quality.

(c) *Lead acetate.*—Dissolve 5 g of U.S.P. Pb acetate in H_2O to make 100 cc.

(d) *Mercurous nitrate.*—U.S.P. $HgNO_3$ test soln.

(e) *Lead triethanolamine.*—Add 1 cc of triethanolamine to a soln of 1 g of U.S.P. Pb acetate in 20 cc of H_2O.

(f) *Barium hydroxide.*—Saturated soln in H_2O.

(g) *Hydrochloric acid.*—0.1 N.

PREPARATION OF SAMPLE

Separate the compound in pure form. Use portions of the purified compound to make solns or to test directly as specified for the individual synthetic.

Controls.—For comparison treat a known sample as directed in the tests.

IDENTIFICATION

To a drop of a soln of the compound or to about 1 mg of the powder on a glass

slide, add a drop of the specified reagent. Do not stir unless directed. Without covering, examine for crystal formation under the microscope, using about 100× magnification. Observe the characteristics of the crystals and compare with controls and description.

Characteristics of microchemical tests for synthetics

SYNTHETIC	FORM	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Diallyl-barbituric acid	Dry powder	—	Lead triethanolamine	Stir a small quantity of the synthetic into a drop of the reagent. Rods singly and in clusters
	Dry powder	—	Barium hydroxide	Stir a small quantity of the synthetic into a drop of the reagent. Rods singly and in groups
Mandelic acid	Water soln	1–100	Lead acetate	Rosettes of thin curving plates
	Water soln	1–100	Mercurous nitrate	Burr-shaped groups of needles
Sulfanilamide	Dry powder	—	Benzaldehyde	Stir thoroly a small amount of synthetic into a drop of reagent. 4-sided plates
	0.1 N HCl soln	Saturated soln	Sodium nitrite	Yellow needles

(4) The following modifications in reagents for synthetics were adopted: On p. 605, change par. 180(b) to read as follows: "Prepare as directed under 26(c)"; change par. 180(h) to read as follows: "Prepare as directed under II, 7(c)"; change par. 180(i) to read as follows: "Prepare as directed under 176(j)"; change par. 180(m) to read as follows: "Prepare as directed under 176(b)."

(5) The status of the methods for the microscopical identification of the synthetic substances named below was advanced from tentative to official (first action):

Acetanilid
Acetophenetidin
Acetylsalicylic acid
Aminopyrine
Amytal
Antipyrine
Barbital
Benzocaine
Benzoic acid

Cinchophen
Dinitrophenol
Methenamine
Neocinchophen
Phenobarbital
Hydroxyquinoline sulfate
Pyridium
Salicylic acid
Triethanolamine

(6) The following method for the determination of hypophosphites in sirups was adopted as tentative:

HYPOPHOSPHITES IN SIRUPS

(Not applicable in the presence of other reducing agents or of phenolic compounds.)

(a) *Bromide-bromate soln.*—Prepare as directed in **26(c)**, p, 551.

(b) *N Sodium thiosulfate.*—0.1 *N*. Prepare as directed in **3(b)**.

(c) *Potassium iodide.*—20 g per 100 cc.

(d) *Sulfuric acid.*—10 g per 100 cc.

(e) *Starch soln.*—0.5 g per 100 cc.

<div align="center">DETERMINATION</div>

Transfer 50 cc of the sirup, measured in a 50 cc volumetric flask, to a 250 cc volumetric flask. Wash the 50 cc flask with several portions of H_2O, adding the washings to the 250 cc flask, finally making up to the mark with H_2O, and mixing well. (This procedure is followed in the case of the sirup of ammonium hypophosphite. For sirups containing larger quantities of hypophosphites the original 50 cc may be diluted to 500 cc in a volumetric flask.) Transfer a 50 cc aliquot to a 250 cc volumetric flask and make up to the mark with H_2O, again mixing well. Of this solution, transfer a 50 cc aliquot to a glass-stoppered 250 cc flask, add 50 cc of the bromide-bromate soln and 20 cc of the H_2SO_4; stopper, shake well, and let stand for 2 hours. Add 10 cc of the KI soln, shake the flask, and titrate the liberated I with the $Na_2S_2O_3$ soln until a straw color appears; then add 2 cc of the starch soln and titrate until the soln becomes colorless. Conduct a blank determination in the same way.

1 cc of 0.1 N $Na_2S_2O_3 = 0.00165$ g of H_3PO_2.

1 cc of 0.1 N $Na_2S_2O_3 = 0.00208$ g of $NH_4H_2PO_2$.

(7) The method published last year (*This Journal*, **21**, 536) for the determination of hexylresorcinol was adopted as tentative.

(8) The method for the determination of methoxyl groups (see p. 100) was adopted as tentative for the evaluation of guaiacol and guaiacol carbonate with the following factors: 1 cc 0.05 N thiocyanate = 1.034 mg of guaiacol; 1 cc 0.05 N thiocyanate = 1.143 mg of guaiacol carbonate.

(9) The following methods for the determinations of acetylsalicylic acid, acetophenetidin, and caffeine were adopted as tentative:

<div align="center">ACETYLSALICYLIC ACID, ACETOPHENETIDIN, AND CAFFEINE</div>

<div align="center">REAGENTS</div>

(a) *Sulfuric acid.*—2%. Pour about 6.0 cc of H_2SO_4 into 500 cc of H_2O.

(b) *Sodium bicarbonate soln.*—Use freshly prepared. Add 3 g of $NaHCO_3$ to 45 cc of H_2O previously cooled to 15°. Stir until dissolved and add 2–3 drops of 10% HCl.

<div align="center">DETERMINATION</div>

Acetylsalicylic acid.—Make this determination as soon as possible to prevent any hydrolysis in the $NaHCO_3$ soln.

Weigh sufficient powdered sample to represent at least 0.04 g of caffeine, transfer to a separator containing about 10 cc of H_2O cooled to 15°, and shake thoroly. Add 15 cc of the cooled $NaHCO_3$ soln slowly to prevent mechanical loss due to effervescence and immediately extract with successive portions of $CHCl_3$. Wash each portion of $CHCl_3$ thru a second separator containing 2 cc of the $NaHCO_3$ soln and filter thru a funnel containing a pledget of cotton moistened with $CHCl_3$. (Extraction is complete when a final shakeout evaporated to dryness leaves a negligible residue. Usually 5 extractions with about 30 cc portions of $CHCl_3$ are sufficient.) Set aside the combined $CHCl_3$ extracts containing the caffeine and

acetophenetidin for later treatment. Transfer the wash H_2O in the second separator to the soln in the first separator, rinsing several times with small portions of H_2O. Acidify the combined $NaHCO_3$ solns with HCl (1 +1) and extract the acetylsalicylic acid by shaking with successive portions of $CHCl_3$, filtering each portion thru a funnel containing a pledget of cotton moistened with $CHCl_3$ (usually 5 extractions are sufficient). Evaporate the combined $CHCl_3$ extracts on a steam bath with the aid of a fan or gentle air blast until the volume is about 10 cc. Transfer to a suitable small tared container with the aid of $CHCl_3$ and evaporate to dryness by means of a fan or gentle air blast without heat. Dry in a desiccator overnight and weigh as acetylsalicylic acid. The extracted acetylsalicylic acid may be checked by the A.O.A.C. bromination method or by the double titration method (p. 551, 27 or 28).

Acetophenetidin and caffeine.—Evaporate the $CHCl_3$ soln containing the acetophenetidin and caffeine on the steam bath and transfer, when the volume reaches 5–10 cc, to a 100 cc beaker by means of small portions of $CHCl_3$. Evaporate again to a volume of about 5 cc and add 10 cc of 2% H_2SO_4. Introduce a stirring rod and heat the mixture on the bath until all the $CHCl_3$ has evaporated, stirring occasionally. Cool to room temp. and decant thru a tared Gooch crucible previously dried to constant wt. at 100°. (No suction is required.) Collect the filtrate in a 150 cc beaker, retaining as much of the acetophenetidin as possible in the beaker. Rinse the sides of the beaker containing the acetophenetidin with 5–10 cc of $CHCl_3$, add 10 cc of 2% H_2SO_4, and heat on the bath as before until all the $CHCl_3$ has evaporated. Cool, and decant thru the same crucible as before. Repeat the process with another 10 cc portion of the H_2SO_4, and finally wash the acetophenetidin quantitatively into the crucible with H_2O. Wash the beaker and crucible with H_2O until the filtrate measures about 75 cc. Dry the crucible at 100° and weigh the acetophenetidin.

To the filtrate containing the caffeine and the small amount of acetophenetidin that went into soln (usually about 0.075 g), add 8 cc of H_2SO_4 (1 +10) and evaporate on the steam bath to a volume of about 10 cc. Transfer by means of small portions of H_2O to a 50 cc Erlenmeyer flask previously marked for volumes of 5 and 10 cc. Proceed as directed in **16, 17**, bearing in mind that the hydrolysis must be continued until no odor of acetic acid is present. The hydrolysis is hastened somewhat if the flask is allowed to hang in the steam from a wire wrapped around its neck so that the mouth of the flask is about level with the surface of the bath. (About 3 evaporations are usually sufficient.) Add the weight of acetophenetidin obtained to the weight of acetophenetidin collected in the Gooch crucible to obtain the total acetophenetidin content of the sample.

(10) The following qualitative tests for the identification of the following gums were adopted as tentative: acacia, agar, galagum, Irish moss, karaya, quince, starch and tragacanth:

IDENTIFICATION OF GUMS

REAGENTS

(a) *Chlorzinc iodide.*—To 100 cc of a soln of $ZnCl_2$, sp. gr. 1.8, add a soln of 10 g of KI and 0.15 g of I in 10 cc of H_2O. (Keep a few crystals of I in the soln.)

(b) *Ruthenium red.*—To a few cc of a 10% soln of Pb acetate add enough ruthenium red to produce a wine red color.

(c) *Methylene blue.*—0.1% soln in alcohol.

(d) *Methylene blue.*—0.1% soln in H_2O.

PREPARATION OF SAMPLES

Controls.—Moisten 1 g of the dry gum with alcohol, add 100 cc of H_2O with constant stirring, and bring to a boil. To 5 or 10 cc of the resulting liquid or jelly, add 4 volumes of 95% alcohol, mix, and centrifuge to bring the precipitate together as a compact mass. (Some gums, notably acacia and agar, may fail to be thrown down by this treatment. The addition of a few drops of a saturated salt soln should cause rapid flocculation and settling.)

Jellies or lotions.—Stir, and add H_2O if necessary to produce a fluid mass. Treat a portion of the sample with 95% alcohol to precipitate the gum as directed under *Controls.* Remove fatty or oily material, if present, by washing the precipitated gum with ether, then redissolve in H_2O and re-precipitate.

PROCEDURE

With a clean towel squeeze a small lump of the alcohol precipitate obtained as directed under *Preparation of Sample* against a microscope slide to form a mat 4–8 mm in diameter on the slide. Note the character of the resulting mat as a possible index to the type of gum. Quince and Irish moss form thin and rather translucent films while agar, starch, and acacia are white and opaque. Cover the mat with a large drop of the chlorzinc iodide soln and observe carefully both with and without magnification. For direct examination place the slide upon a white surface. For microscopical examination use a magnification of about 90 diameters. If no characteristic color is produced within 1–2 min, proceed with a fresh mat to examine for the following group. Continue in a similar manner through all the group tests or until the identity is established. Use a fresh mat for each individual test.

Characteristics of tests for gums

Group I.—Reagent Chlorzinc iodide

GUM	ORIGINAL ALCOHOL PPT.	GROUP REACTION	CONFIRMATORY TEST	REMARKS
Tragacanth	Stringy bluish Translucent	Blue color	Warm with 10% NaOH on steam bath Yellow color	Certain gums, e.g., Irish moss, may yield dull yellow color with NaOH. Tragacanth bright yellow
Starch	White Compact	Blue black color	Iodine, 0.1 N Blue color	Tragacanth may yield faint blue
Quince	Stringy Translucent	Blue color	Above tests negative	Quince is distinguished from starch and tragacanth by negative reactions
Irish moss	Stringy	Brown (small blue particles)	Characteristic nodular structures with group reagent	Old preparations of this gum may fail to show characteristic structures

Group II.—Reagent Tincture of Iodine U.S.P.
(Allow tincture to dry on mat, flush off with 95% alcohol, and irrigate with water.)

Agar	White opaque	Opaque blue black	Stains with Ruthenium red	Does not dissolve or lose shape when covered with H_2O
Irish moss	Stringy	Brown or lilac	Characteristic blue stain with alcoholic methylene blue	These reactions yielded by old as well as fresh preparations

Group III.—Reagent Ruthenium Red

Karaya	Fine flocculent compact mass on centrifuging	Swells considerably Strongly stained pink granular mass	Heat with conc. HCl. Pink color	Aqueous methylene blue produces a characteristic blue stain

Group IV.—Reagent Concentrated H_2SO_4
(Warm cautiously on steam bath.)

Galagum	Stringy	Pink or red brown color	No satisfactory test found	The alcohol precipitate from galagum resembles that from tragacanth
Acacia		Greenish brown	Ppt. completely soluble in H_2O	The complete soln of acacia distinguishes it from most other gums

(11) The present tentative method for the determination of theobromine in theobromine calcium (p. 590, 137) was retained in that status, and the following method was adopted as a tentative, alternative method:

THEOBROMINE IN THEOBROMINE CALCIUM—TENTATIVE

INDICATOR

Phenol red.—Triturate 0.1 g of phenol red in an agate mortar with 15 cc of 0.02 N NaOH until dissolved and dilute the soln with recently boiled H_2O to 200 cc.

DETERMINATION

Place 0.5 g of the powdered tablets, previously dried at 110°, or 0.4 g of theocalcin powder, or 0.2 g of theobromine alkaloid in a 300 cc beaker and add 100 cc of H_2O. Warm moderately over a flame and add 15 cc of approximately 0.1 N H_2SO_4. Heat to boiling to insure complete soln and to remove CO_2. Cool to room temp. Add 1.5 cc of phenol red indicator and render slightly alkaline with approximately 0.1 N H_2SO_4 (yellow color). To this soln add 25 cc (an excess) of neutral 0.1 N $AgNO_3$ and titrate the liberated HNO_3 immediately with 0.1 N NaOH to a distinctly violet red color. Titrate cautiously drop by drop with constant stirring near the end point.

1 cc of 0.1 N NaOH = 0.018 g of $C_7H_8O_2N_4$.

(12) The following method for the determination of chlorobutanol (chloretone) was adopted as tentative:

CHLOROBUTANOL

REAGENTS

·(a) *Alcoholic potassium hydroxide soln.*—Prepare as directed in 106(a).

(b) *Silver nitrate soln.*—Dissolve 10 g of AgNO$_3$ in sufficient H$_2$O to make 500 cc.

DETERMINATION

Transfer to a pressure bottle a sample equivalent to about 0.3 g of chlorobutanol and carefully add 25 cc of the alcoholic KOH soln. Stopper the bottle, and mix the contents by gentle swirling, taking care to prevent the soln from coming in contact with the rubber washer, then allow to stand 30 min. or overnight. Place the bottle in a wire basket, and set the basket in a water bath at room temp. Invert a tin can over the bottle and cover with a towel to prevent injury in case the bottle should burst. Heat the bath to boiling and maintain at this temp. for 15 min.

Cool gradually; add 25 cc of H$_2$O, swirling gently; and transfer the contents of the pressure bottle to a 400 cc beaker. Wash the bottle with H$_2$O, draining the washings into the beaker. Add 15 cc of HNO$_3$, an excess of the AgNO$_3$ soln, stir well, and allow the mixture to stand in a dark place for 15 min. Collect the precipitate in a Gooch crucible that has been dried at 105° and weighed. Wash the precipitate thoroly with distilled H$_2$O, then with 5 cc of alcohol followed by a 5 cc portion of ether. Dry to constant weight at 105°. If reagents contain Cl, apply correction determined thru a blank test. 1 g of AgCl = 0.4127 g of C$_4$H$_7$OCl$_3$.

In ampoule solns.—Pipet into a distilling flask a sample equivalent to about 0.1 g of chlorobutanol. Add sufficient H$_2$O to bring the volume to 50 cc and distil about 25 cc thru a straight-bore condenser. Collect the distillate in a pressure bottle of approximately 100 cc capacity containing 25 cc of the alcoholic KOH and surrounded by an ice bath. Have the delivery tube extend into the alcoholic soln. (It is essential that a straight-bore condenser be used to assure complete soln of the crystals of chlorobutanol in the condenser.) Allow to cool, disconnect the still head, and wash the condenser carefully with 25 cc of alcohol, allowing the alcohol to drain into the pressure bottle. Repeat the washing, using about 20 cc of H$_2$O. Also wash the receiving tube with H$_2$O.

Stopper the pressure bottle and mix the contents by gentle swirling, taking care to prevent the soln from coming in contact with the rubber washer. Allow to stand 30 min. or overnight. Complete the determination of Cl as directed above.

(13) The following method for the separation of acetylsalicylic acid and phenolphthalein was adopted as tentative:

ACETYLSALICYLIC ACID AND PHENOLPHTHALEIN IN TABLETS

PREPARATION OF SAMPLE

Count and weigh a representative number of tablets and calculate the average weight. Powder finely in a mortar and keep in a tightly stoppered bottle.

DETERMINATIONS

Weigh sufficient of the powdered material to contain from 0.05 to 0.1 g of phenolphthalein. Extract the dry powder repeatedly with 20 cc portions of ether and filter into a separator. Test for complete extraction (5–8 extractions required).

Acetylsalicylic Acid.—Shake the ethereal soln for at least 1 min. each time with two 20 cc portions of 4% NaHCO$_3$ soln (temp. 20° or less). Transfer the soln to a second separator. Wash the ether with two 10 cc portions of H$_2$O and add to the

bicarbonate soln. Extract the bicarbonate soln with 20 cc of ether. Draw off the lower aqueous layer into a 100 cc volumetric flask. Wash the ether with small portions of H_2O, rinse into the flask, and dilute to the mark. Add the wash ether to the bulk of the solvent in the original separator. Reserve the ethereal soln for the determination of the phenolphthalein.

Transfer an aliquot of the bicarbonate soln containing not less than 0.3 g of acetylsalicylic ac d to a separator. The acid must be isolated from the bicarbonate soln as rapidly as possible to prevent hydrolysis. Acidify with 10% HCl and extract the liberated acetylsalicylic acid with a 3 +2 $CHCl_3$-ether mixture (30, 20, 20, 10, and 10 cc fractions). Wash each extraction with 2 cc (used for all extractions) of H_2O in a second separator and filter thru a pledget of cotton moistened with the solvent into a counterpoised tared beaker. Test for complete extraction. Evaporate the solvent to a volume of 10–15 cc on the H_2O bath and complete the evaporation without the aid of heat. Dry the residue to constant weight at room temp. The weight may be checked by the double titration method (28).

Phenolphthalein.—Extract the original ethereal soln with 20 cc portions of 3% NaOH soln until all the phenolphthalein has been removed as indicated by the color. Transfer these alkaline extracts to a second separator, acidify with 10% HCl, and extract with $CHCl_3$-ether solvent. Wash each portion of solvent in a third separator with 2 cc of H_2O to which has been added 1 or 2 drops of 10% HCl. Filter the extracts into a counterpoised tared beaker, using in the stem of the funnel a pledget of cotton moistened with the solvent. Evaporate the solvent on the H_2O bath and dry the residue to constant weight at 120°. The weight may be checked by the tetraiodo method (p. 569, 78).

(14) The following method for the determination of cod liver oil in emulsions was adopted as tentative:

COD LIVER OIL IN EMULSIONS

Weigh into a tared beaker of about 150 cc capacity sufficient of the well-mixed sample to contain about 2 g of cod liver oil. Add about 10 g of finely powdered $CaCO_3$ and thoroly mix with a stirring rod. Add 30 cc of $CHCl_3$, thoroly mix, and decant thru a dry filter into a 100 cc air-dried, tared beaker. Continue to extract and wash repeatedly with 5–10 cc portions of $CHCl_3$ until the filtrate is about 60 cc. Evaporate the $CHCl_3$ on a steam bath with a current of air to about 5 cc.

Continue extraction and carefully wash the filter paper and funnel, filtering into a 250 cc beaker until the filtrate is about 150 cc. Evaporate to about 10 cc and transfer to the first tared beaker. Repeat the procedure until extraction is complete or until 25 cc of the solvent upon evaporation in a second tared beaker yields 0.001 g or less of residue.

Evaporate the $CHCl_3$ in the first tared beaker and allow to remain on the steam bath for about 10 min. after the odor of $CHCl_3$ has disappeared. Dry in the oven at not over 100° for 5 min. intervals until weight is constant or the loss is 0.001 g or less.

CAUTION: Avoid prolonged heating or long exposure to air at room temp. The oil absorbs oxygen, the weight increases appreciably, and the physical constants change.

(15) The following method for the determination of mercury in ointment of mercuric nitrate was adopted as tentative:

MERCURY IN OINTMENT OF MERCURIC NITRATE (CITRINE)

Transfer to a 200–300 cc Erlenmeyer flask 3–5 g of the sample accurately weighed, using a glass or bone spatula. Add 40 cc of HNO_3 (1 +1) and a few glass beads and insert a short-stemmed funnel into the neck of the flask. Boil gently 1–1.5 hours on

a hot plate or over a low flame. With the latter use a piece of asbestos having a circular hole under an asbestos wire gauze. Add 30 cc of H_2O, using a part to wash the funnel. Cool sufficiently to cause the unconsumed fat to form a hard cake (approx. 20° or below). Filter thru an 11 cm filter into a 200 cc volumetric flask. Wash the fat, flask, and filter, using about 100 cc of 1% HNO_3. Make to volume and mix well. Reserve the fat for test for complete extraction as directed below.

Transfer a 100 cc aliquot to a 500 cc Erlenmeyer flask. Add 7 cc of HNO_3, 5 cc of H_2SO_4, and 2 g of powdered permanganate, and rotate to dissolve. Heat just to boiling over a low flame or on a hot plate. Boil gently 45 min., maintaining an excess of permanganate, indicated by a dark purple color. (The presence of an excess throughout this period is essential.) When adding permanganate to the boiling liquid use smaller portions (approx. 0.5 g or less) to avoid loss due to frothing. (The use of a greater excess of permanganate than is necessary is not objectionable except that it will require proportionately more of the peroxide to remove it and the MnO_2 at the end of the digestion. Usually about 10 g is required.)

CAUTION: The rate of consumption and total permanganate consumed seem to vary with the temp., the organic matter present, and the period of heating. Furthermore, the large amount of MnO_2 formed may lead to the wrong conclusion concerning the color indicative of an excess of permanganate. Frequent examination of the soln is necessary. The observation of this color is aided by looking through the supernatant liquid toward a white background while holding the container in an inclined position.

Remove excess permanganate and dissolve MnO_2 by adding H_2O_2 (5–10% prepared from 30%) dropwise to the hot soln. When colorless add 2% $KMnO_4$ soln slowly until a faint pink or brown persists for about 1 min. If a large amount of MnO_2 forms at this point, use the peroxide sparingly again, then permanganate to discharge the peroxide. Discharge the color from the last permanganate, including a weak brown color from MnO_2, by adding dropwise just sufficient ferrous sulfate T.S. Cool to about 20°, add 3 cc of ferric $(NH_4)_2SO_4$ T.S. and titrate with the standard thiocyanate.

1 cc of 0.1 N thiocyanate $= 0.01003$ g of Hg.

Test for complete extraction of the Hg from the fat and its removal from the filter, etc., by repeating the $1+1$ HNO_3 digestion for about 30 min. on the residual fat in the flask or on the filter, completing this as a separate determination, including the permanganate digestion. Add any titration in excess of 1 to 2 drops (approximately 0.05 to 0.08 cc) of 0.1 N NH_4CNS resulting from this test portion to that obtained by titrating the main extract.

(16) The following method for the determination of sulfanilamide was adopted as tentative:

SULFANILAMIDE

Place on a 9 cm folded filter paper in a funnel a portion of the sample containing about 0.5 g of sulfanilamide. Wash the soluble portion with a fine stream of acetone into a 250 cc flask, using a total of about 25 cc of acetone. Test for complete extraction by evaporating a small portion of the washings. Immerse the flask in a H_2O bath at about 70° until the acetone has been evaporated and its odor is no longer perceptible. Remove from the bath and add 10–12 cc of 75% (by volume) H_2SO_4. Connect the flask to a reflux condenser with water jacket, add a few glass beads, and boil the soln slowly for 30 min. Wash down the condenser with H_2O, make the liquid in the flask to about 100 cc with H_2O, add an excess of 50% alkali, distil, and collect the ammonia in the distillate in an excess of 0.1 N H_2SO_4. Titrate the excess acid with 0.1 N NaOH, using methyl red indicator.

1 cc of 0.1 N $H_2SO_4 = 0.01722$ g of $(NH_2)_2C_6H_4SO_2$.

(17) The following methods for the qualitative and quantitative determination of mandelic acid were adopted as tentative:

MANDELIC ACID

Qualitative Tests

(Applicable to the free acid)

(a) Dissolve 0.25 g of the sample in about 10 cc of H_2O and add a few drops of 10% $FeCl_3$ soln. A bright yellow color is produced. This is a general test for hydroxy acids and is not specific for mandelic acid.

(b) Dissolve 0.25 g of the sample in 5 cc of H_2O in a test tube; to the soln add 5 cc of H_2SO_4 and agitate the test tube and contents for a few seconds; then add 10 cc of H_2SO_4 so as to form two layers. Agitate very gently but do not mix. A purple color slowly forms at the interface if the test tube is allowed to stand for a few minutes. A strong odor of benzaldehyde is noticed on shaking.

Quantitative Methods

Tablets.—Count and weigh a representative number of tablets, ascertain the average weight, and grind to a fine powder. Weigh a quantity of the powdered material equivalent to from 0.4 to 0.5 g of mandelic acid and transfer to a separator containing 10 cc of H_2O. Acidify with HCl (1+3) and add 2 cc of the acid in excess. Extract with six 20 cc portions of chloroform-ether solvent (2+1); wash each portion in a second separator with 2 cc of H_2O, and pass the soln thru a plug of cotton, previously saturated with the solvent, into a 250 cc beaker. Wash the outer surface of the stem of the separator with a few cc of solvent and add this to the main portion. Test for complete extraction with 15 cc more of solvent and evaporate in a separate beaker. Wash any residue thus obtained into the beaker containing the main extract with a few cc of solvent.

Evaporate to dryness at a temp. not exceeding 40° with the aid of a fan. Dissolve the residue in 25 cc of CO_2-free distilled H_2O and titrate with 0.1 N NaOH, using phenolphthalein as indicator.

1 cc of 0.1 N NaOH = 0.01521 g of mandelic acid ($C_6H_5CHOHCOOH$), 0.01691 g of NH_4 mandelate ($C_6H_5CHOHCOONH_4$), 0.01741 g of Na mandelate ($C_6H_5CHOHCOONa$), 0.01711 g of Ca mandelate ($C_6H_5CHOHCOO)_2Ca$, or 0.01632 g of Mg mandelate ($C_6H_5CHOHCOO)_2Mg$.

After titration the mandelic acid may be re-extracted and the extract used for melting point determinations or qualitative tests.

Liquid preparations.—Measure 1 cc of the sample or such amount of an aliquot of a dilution sufficient to yield from 0.4 to 0.5 g of mandelic acid into a separator and acidify with HCl(1+3). Proceed as directed above for tablets.

(18) The official method for the determination of camphor (p. 560, 51) was amended (first action) by the insertion, between the title and the text, of the following expression in parentheses: "(Not applicable to synthetic camphor)."

(19) The method for the determination of bismuth compounds in tablets (p. 592, 143) was amended by the deletion of the expression "(Lead Absent)" and the substitution of the expression, "Not applicable in the presence of lead compounds but applicable in the presence of cerium salts." The method was further amended by the insertion before the present text of the following paragraph:

Count and weigh a suitable number of tablets and ascertain their average weight. Pulverize the tablets and preserve the powder in a tightly stoppered bottle.

(20) The last paragraph in Method II (p. 576, 97) was transposed to constitute a second paragraph in Sec. 96, p. 576, and the status of this paragraph was advanced to that of official (first action).

(21) The directions for preparing reagents in the microchemical tests for alkaloids and synthetics (p. 602, 176; p. 605, 180) were amended as follows:

(1) The words "Potassium thiocyanate" in par. 180(1) were changed to read "Ammonium thiocyanate."

(2) The reagent for ethylhydrocupreine, "potassium thiocyanate" (e) (*This Journal*, 20, 80) was changed to read "ammonium thiocyanate."

(3) The directions for preparing Wagner's reagent (p. 543 5(b) and p. 605, 180(d)) were deleted and the statement, "Prepare as directed in 176 (c)" was substituted.

(22) The tentative method for the determination of chloroform in mixtures (p. 579, 105) was amended as follows:

(1) The quantity of calcium carbonate used as a reagent was changed from 1.0 g to 0.1 g.

(2) The use of carborundum chips to prevent bumping was permitted.

(3) The term "pressure bottle" was substituted for that of "citrate bottle."

(4) The following caution was inserted:

CAUTION: Do not cool the pressure bottle suddenly. It is best to allow it to cool in the H_2O in which it was boiled.

(5) The reagent, alcoholic potassium hydroxide, 104 (a), was deleted, and the reagent alcoholic potassium hydroxide, 106 (a), was substituted therefore.

(6) The method (after amendments) was retained in its tentative status.

(23) The official method for the determination of barbital and phenobarbital (p. 582, 112) was amended (first action) by the addition at the end of par. 112 of the following expression: "Determine the melting point to check the purity of the residue."

(24) The methods for the determination of phenolphthalein (p. 569, 77, 78) were amended as suggested by the associate referee as follows:

(1) In par. 77, line 1, change "0.1 g" to "0.2 g" (first action).

(2) In par. 79, line 4, p. 570, after "10 cc each," insert the expression "(or sufficient to represent about 0.2 g of phenolphthalein)."

(3) In par. 80, line 4, p. 570, delete the sentence, "Then make alkaline ... the process three or four times," and substitute the following sentences:

If sufficient I has been added, the precipitate, as well as the supernatant liquid, will be brown; if not, add more I to insure an excess, and then the strong KOH soln

dropwise, with stirring, to dissolve the precipitate completely and consume all the excess I. (This soln should be blue or blue-purple.) Repeat the process of precipitation with strong acid and resolution with strong alkali 3 or 4 times with small quantities of the reagents, adding small pieces of ice if necessary to keep the soln cold. In the acid condition there should be a brown precipitate resembling a periodide, and the supernatant liquid should be colored brown by the excess I. (The alkaline soln should be clear blue or purple-blue, and no precipitate should be present.)

(25) The method for the determination of thymol (p. 571, **84**) was adopted as official (final action).

(26) The tentative methods for the assay of the following drugs were adopted as official (first action): Aloin (p. 567, **75**); barbital and phenobarbital (applicable in presence of stearic acid), (p. 582, **113**); dinitrophenol and its sodium compound (*This Journal*, **20**, 82); ether (p. 584, **120**); homatropine in tablets (*This Journal*, 21, **95**); iodoform and iodoform gauze (p. 594, **148, 150**); morphine in sirups (p. 586, **125**); phenolsulfonates (p. 597, **161**); thymol in antiseptics (p. 572, **85**); and santonin in mixtures (p. 588, **129**).

XL. BACTERIOLOGICAL METHODS

No additions, deletions, or other changes.

XLI. MICROCHEMICAL METHODS

The following method proposed by the referee for the determination of methoxyl (*This Journal*, **20**, 292), was adopted as tentative:

METHOXYL AND ETHOXYL GROUPS

REAGENTS

(a) *Acetic acid-potassium acetate soln.*—Dissolve 10 g of K acetate in sufficient glacial acetic acid to make 100 cc of soln.

(b) *Sodium acetate soln.*—Dissolve 25 g of crystalline Na acetate in sufficient H_2O to make 100 cc of soln.

(c) *Approximately 0.05 N thiosulfate soln.*—Boil 2.5 liter of H_2O until 1/5 has evaporated, cool to about 75°, and then add the necessary thiosulfate and 20 cc of amyl alcohol (byproduct from alcoholic fermentation). Allow to cool and standardize against a standard KIO_3 soln.

DETERMINATION

To 5 cc of the K acetate soln, add 15 drops (ca. 0.2 cc) of Br_2, and place 2/3 of this liquid in receiver C and the remainder in D. Then weigh approximately 20 mg of substance upon a tared piece of cigarette paper (15×25 mm) and place both the paper and the contents in the bottom of boiling flask A, together with a boiling rod. (A glass tube approximately 60 mm long, 3.5 mm o.s. diameter with a 1 mm bore. It is sealed at one end and also closed about 10 mm from the other. The open end is fire polished. When this rod is placed in the flask with the open end down it will cause uniform boiling indefinitely if sufficient heat is constantly applied to the flask.) Add 2.5 cc of melted phenol from a wide-tipped pipet and 5 cc of HI and then connect the flask to the remainder of the apparatus, which consists of the trap (B), containing a little H_2O, and the receivers C and D. Pass CO_2 thru the apparatus

from the capillary side arm of the boiling flask at a uniform rate of 15 cc per min., and boil the liquid by means of a mantled micro burner at such a rate that the vapors of the boiling liquid rise about half way in the air condenser. Continue the boiling for 30–60 min. (If the type of substance is known to require only 30 min., this
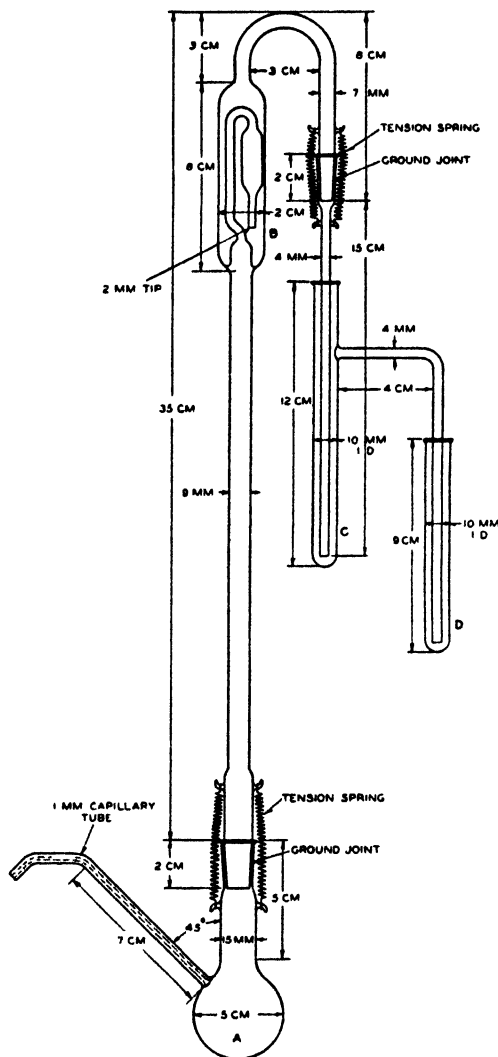
SEMI-MICRO ZEISEL METHOXYL APPARATUS

period should be used, but for materials about which such information is lacking an hour should be used as a general procedure.) Disconnect the apparatus and wash the contents of the receivers into a 250 cc Erlenmeyer flask containing 5 cc of Na acetate soln. Adjust the volume of the liquid to 100 cc and reduce the excess Br_2 with formic acid (approximately 15 drops is sufficient).

Remove any Br_2 vapor in the flask by drawing air over the liquid from a vacuum

line or by blowing air over the liquid, then add 0.5 g of KI and 5 cc of 10% H_2SO_4 soln. Titrate the liberated I with the thiosulfate soln, using starch as an indicator.

Obtain the blank on all the reagents by making a determination without a sample and subtract this from the quantity of thiosulfate soln used when the sample was present. 1 cc of 0.05 N thiosulfate = 0.2586 mg of methoxyl (OCH_3).

The same procedure applies to ethoxyl groups. 1 cc of 0.05 N thiosulfate = 0.3754 mg of ethoxyl (OC_2H_5).

XLII. REFERENCE TABLES

No additions, deletions, or other changes.

APPENDIX I. STANDARD SOLUTIONS

(1) The following method submitted by the referee for the standardization of acid solutions with borax was adopted as official (first action).

STANDARDIZATION OF ACID SOLUTIONS WITH BORAX[1]

REAGENTS

(a) *Methyl red indicator.*—Dissolve 100 mg of methyl red in 60 cc of alcohol and dilute with H_2O to 100 cc.

(b) *Sodium borate.*—U.S.P. quality or better and should pass the following purity tests:

(1) *Insoluble impurities soln of 5 g of salt in 95 cc of warm H_2O.*—Should be clear and colorless.

(2) *Chloride.*—20 cc of 5% soln must not give an opalescence with HNO_3 and $AgNO_3$ that is stronger than 20 cc of a Cl soln that has a strength of 5 mg of Cl per liter.

(3) *Sulfate.*—20 cc should give no precipitate with acetic acid and $BaCl_2$ after standing 30 min.

(4) *Calcium.*—20 cc of the hot soln should give no turbidity with NH_4 oxalate after cooling.

(5) *Magnesium.*—20 cc of soln must not give any microcrystalline precipitate with ammonia and phosphate after standing 24 hours.

(c) *Reference soln.*—Prepare a reference soln of boric acid, NaCl, and indicator corresponding to the composition and volume of the soln at the equivalence point. For use in the determination of the end point of a titration with 0.1 N acid, the reference soln should be 0.1 M in boric acid and 0.05 M in NaCl.

(d) *Standard borax.*—Saturate 300 cc of H_2O at 55° (not higher) with borax (approximately 45 g). Filter at this temp. thru a folded filter into a 500 cc Erlenmeyer flask. Cool the filtrate to approximately 10°, with continuous agitation during the crystallization. Decant the supernatant liquid. Rinse the precipitate once with 25 cc of cold H_2O. Dissolve the crystals in just enough H_2O at a temp. of 55° to insure complete soln (approximately 200 cc). Re-crystallize by cooling to approximately 10°, agitating the flask during crystallization. Filter the crystals onto a small Büchner funnel with suction. Wash the precipitate once with 25 cc of ice-cold H_2O. Dry the crystals[2] by washing with two 20 cc portions of 95% alcohol, drying after each washing with suction. Follow with two successive 20 cc portions of U.S.P. ether. Spread the crystals on a watch-glass, and set aside for about 12 hours in order that the last traces of ether may evaporate. Protect the borax from dust. Allow the $Na_2B_4O_7 \cdot 10H_2O$ to stand about 12 additional hours in a desiccator over a soln saturated with respect to both sugar and salt before use. Then transfer the pure borax into a container that has a ground-glass stopper and store in the desiccator when not in use (stable under these conditions for 1 year).

[1] Kolthoff, *Volumetric Analysis*, II, 93–96 (1929).
[2] Hurley, F. H., *Ind. Eng. Chem. Anal. Ed.*, **8**, 220 (1936).

Accurately weigh sufficient of the standard borax to titrate approximately 40 ml and transfer to a 300 cc flask. Add 40 cc of CO_2-free H_2O and stopper the flask. Swirl gently until the sample is in soln. Add 4 drops of the methyl red indicator and titrate with the soln that is being standardized to the equivalence point as indicated by the reference soln. Calculate the normality (N) of the standard soln by the following formula:

$$N = \frac{\text{g of } Na_2B_4O_7 \cdot 10H_2O}{\text{ml of acid} \times 190.72/1000}.$$

(2) The following method for the standardization of acid solutions with sodium carbonate was adopted as official (first action).

STANDARDIZATION OF ACID SOLUTIONS WITH SODIUM CARBONATE

REAGENTS

(a) *Methyl orange indicator.*—0.1% of H_2O.

(b) *Sodium bicarbonate.*—C.P. Should pass the following tests for purity:

(1) *Chloride.*—0.5 g of $NaHCO_3$ dissolved in 10 cc of 2 N HNO_3 (free of Cl). With $AgNO_3$ must give no opalescence.

(2) *Sulfate.*—0.5 g of $NaHCO_3$ in 10 cc of 2 N acetic acid. After the addition of $BaCl_2$ should give no turbidity or separation of $BaSO_4$ after standing 15 min.

(c) *Reference soln.*—80 cc of CO_2-free H_2O with 3 or 4 drops of methyl orange indicator.

(d) *Anhydrous sodium carbonate.*[1]—Heat 250 cc of H_2O to 80° and add $NaHCO_3$, stirring until no more dissolves. Then filter the soln thru a folded filter (the use of a hot water funnel is desirable) into an Erlenmeyer flask. Cool the filtrate to about 10° with constant swirling during crystallization. The fine crystalline trona and bicarbonate that separates out has the approximate composition: Na_2CO_3; $NaHCO_3$, $2H_2O$. Pour off the mother liquor. Drain the crystals by suction and wash once with cold H_2O.

Transfer the precipitate, being careful not to include any fibers of filter paper, into a large flat-bottomed dish. Heat in an electric oven with a pyrometer control at a temp. of 290° for 1 hour. Stir the contents occasionally with a Pt wire. After heating, cool the Pt dish and contents in a desiccator. Store the anhydrous Na_2CO_3 in a container having a ground-glass stopper and in a desiccator containing a good desiccant. Dry the salt at 120° just before using.

STANDARDIZATION

Accurately weigh sufficient anhydrous Na_2CO_3 to titrate approximately 40 ml and transfer to a 300 cc Erlenmeyer flask. Add 40 cc of H_2O to dissolve the salt. Add 3 drops of the methyl orange indicator and titrate[2] until the color begins to deviate from the H_2O tint (reference soln). (The equivalence point has not been reached.) Boil the soln gently 2 min., then cool. Titrate until the color is barely different from the H_2O tint (of the indicator).

Calculate the normality (N) of the standard soln by the following formula:

$$N = \frac{\text{g of } Na_2CO_3}{\text{ml of acid} \times 53/1000}.$$

[1] Kolthoff, *Volumetric Analysis*, II, 88 (1929).
[2] *Ibid.*, p. 86.

(3) The tentative methods for the preparation and standardization of solutions of sodium hydroxide (681, 1) were adopted as official (first action).

(4) The tentative methods for the preparation and standardization of hydrochloric acid solutions (682, 5) were adopted as official (first action).

APPENDIX II. DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

See p. 45 for the definitions adopted as official this year.

No report was given by the Committee on Standard Scale for Immersion Refractometer.

REPORT OF COMMITTEE TO COOPERATE WITH THE AMERICAN PUBLIC HEALTH ASSOCIATION ON METHODS OF MILK ANALYSIS

At the 1937 meeting of this Association your Committee reported that a revision of the chemical section of "Standard Methods of Milk Analysis" of the American Public Health Association for the 7th edition of that text was practically completed; it remained only to make such changes or additions as were adopted at that meeting touching the subject matter of the revised text. These changes were made and the manuscript duly forwarded.

Publication of the revision by that Association has been delayed, and the text is not yet published. It will probably be thought advisable to make such further changes in the manuscript as may be necessitated by actions taken at our sessions just ending.

In addition to these periodic revisions your Committee deems it to be within the intent and purpose of its work to advise with members and committees of the American Public Health Association to the end that, not only the methods of analysis for milk and cream, but those for other commodities in which that association and ours have a mutual interest shall be the same in the two groups. The desirability of such uniformity is too obvious to need elaboration. What appears to be a sound and workable plan is already in operation. It permits of three procedures.

1. A mutual transfer of methods from one association to the other in case of methods that are already accepted by one or the other body, and where further work of a collaborative nature is unnecessary.

An illustration of this procedure is the transfer of our methods for milk and cream to the A.P.H.A. book of methods without change and the reciprocal transfer of the A.P.H.A. method for visible dirt in milk from their methods to ours.

2. Because of overlapping membership in the two associations it is sometimes possible to have one and the same referee serve both groups.

An illustration of this is the case of our General Referee on Vitamins, who serves both associations in that capacity.

3. A general referee in the A.O.A.C. may have associates and/or collaborators in the A.P.H.A.

An instance of this plan of procedure is the adoption of the phosphatase test by the A.O.A.C. last year, the test being developed primarily by an A.P.H.A. worker, but duly appointed as an associate to our General Referee on Dairy Products.

In all of these modes of operation the rules of the A.O.A.C. governing the adoption of methods are followed strictly. Transfers or recommendations for adoption of methods are subject to the consideration of the A.O.A.C. referee concerned, thereafter to review and approval or disapproval by the Committee on Recommendations of Referees, and finally to action of our association. Moreover, there is no conflict with the rules of the A.O.A.C. in the appointment of A.P.H.A. members as referees, associates or collaborators because such members are, by virtue of their work and office, potential members of our association.

<div style="text-align: right">E. M. BAILEY
F. C. BLANCK
G. G. FRARY</div>

Approved.

REPORT OF REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE

The annual address of the President (Dr. C. C. McDonnell) of this Association in 1937 was devoted to a discussion of the "Role of Chemistry in Combating the Insect Menace." The facts presented in this address emphasized quite strongly the importance of chemistry in the protection of crops from insect ravages. Coupled with the fact that chemistry plays an equally important role in the protection of crops from plant diseases and also with the fact that this Association's work was really responsible for the inauguration and development of insecticide and fungicide chemistry, you can appreciate the reasons for this Association's connection with the Crop Protection Institute. In fact, it might also indicate that this Association has some obligation to actively promote chemistry in the Institute's program.

The role played by a balanced and proper nutrition of plants on the prevention of disease and insect attacks emphasizes the importance of chemistry in the control program. This, coupled with the relationship that well nourished crops bear to the health of the consumer, makes these problems of vital concern to this Association.

Many of the Institute's research projects pursued in recent years were inspired by the desire of both professional and commercial interests to

find insecticides and fungicides that would be harmless to the consumers of food products and meet the tolerance permitted by the Federal and State Boards of Health. The search for products to conform to these specifications has presented for tests and chemical examinations many extracts, organic and synthetic compounds, and factory by-products.

In addition to the chemical studies of these materials required for the direct and immediate objective, the studies in many instances have necessitated biochemical investigations as to the physiological effects that the applications or sprays may produce on the plant and also as to the effect the soil accumulations of the residues may have on plant nutrition.

The Crop Protection Institute has two classes of projects in progress; viz: regular and exploratory or preliminary.

The preliminary or exploratory projects are undertaken for the purpose of determining if a material is worth more extensive study, and not with any idea of appraising its value. In many instances the preliminary study uncovers promising materials, and in others the results show that it would be unwise to expend more time or expenses in pursuing it further. The Institute has conducted exploratory projects during the past year for twenty-one commercial organizations. Some of these have developed into regular projects.

ACTIVE, REGULAR, ORGANIZED PROJECTS

During the past year the Institute has conducted regular projects for eight commercial organizations. In some instances there have been several projects for one company.

The Institute plans the project, selects the leader, and locates the work with the object of procuring, in the maximum degree, results which will advance some scientific knowledge and be a help and guide to the supporting company.

Dr. W. C. O'Kane, the Director of the Institute, should be commended for the high research standards and policy that have been established and followed by the Institute and for the worth-while results and scientific contributions which it has made.

We recommend that the members of this Association cooperate wherever possible in promoting the Crop Protection Institute's program, and make chemistry a real force in solving the many problems presented for solution.

<div align="right">

H. J. PATTERSON
W. H. MACINTIRE
</div>

Approved. _____

REPORT OF SECRETARY-TREASURER

Again we have broken the record in regard to attendance at our meetings, with a registration of 553.

Last Spring before my trip to Europe I called the Executive Committee together, and Miss Lapp was made Acting Secretary and Treasurer in my absence. The deposit box for the Association's bonds (all of these are registered bonds) was in Miss Lapp's name personally. There was a desire to change this arrangement, which was done, and the Association box (which is now quite a valuable box) was registered in the name of the Association of Official Agricultural Chemists, Incorporated, with Miss Lapp as custodian.

During the year the President, on the advice of the Executive Committee, made one appointment, that of K. L. Milstead, Associate Referee on Iodine and Thiosulfate Solutions. Owing to retirement from public work, Dr. J. F. Snell of Canada submitted his resignation. He was Associate Referee on Maple Products.

Shortly after the meeting of 1937 the Association suffered a serious loss in the death of Dr. Richard Brackett, a loyal member of the Association for many years. The obituary was written by Dr. MacIntire, and this was published in the May, 1938, number of *The Journal*. Three other valuable members died during the year: Dr. Street, Dr. Chesnut, and Dr. Cavanaugh. All of you knew Dr. Street and Dr. Chesnut. Dr. Cavanaugh was head of the Agricultural Chemistry Department at Cornell but had not been active in the work of the Association in recent years. Later Dr. Browne will present comments on these departed members.

The usual routine business was transacted at the meeting of the Executive Committee. On matters other than those contained in the report of the Treasurer it seems unnecessary to go into much detail here. One matter that I should like to report to you is the action taken by the Committee on the splendid report and recommendation of a committee appointed last year to outline a plan for a memorial to Dr. Wiley. This project has been talked about for a number of years. The memorial lectures were conceived first. These lectures will require one more year to complete a series of ten, and it is the purpose of the Committee on completion to bind the reprints into a volume for distribution among the members.

However, since the membership of the Association changes it was thought wise to change the type of lectures and to plan for another memorial to the man who was the founder of this organization. This committee was instructed to bring in a plan or plans for fellowships, memorial fellowships in agricultural chemistry. The committee, composed of Dr. W. H. MacIntire, Chairman; Dr. W. B. White, and Dr. E. M. Bailey, submitted to the Executive Committee two plans, one for an undergraduate scholarship endowment and one for a graduate scholarship endowment. The Committee, after much serious debate, decided to adopt and approve the recommendations for the undergraduate scholarships. The report of the Fellowship Committee in regard to these awards will follow my report.

I will say that we have the money to meet this obligation, and that the Committee considers this project one of stimulating interest in the field of agricultural chemistry, which is important to this Association. Mr. Chairman, I recommend the approval of this action by the Executive Committee. I think it would be quite appropriate if you ask that this report be accepted and approved. (Approved.)

I shall present at this time the financial part of the report of the Secretary-Treasurer. As I have told you before, some four years ago the business affairs of the Association had reached a point where it was found necessary to incorporate the Association of Official Agricultural Chemists. The officers elected now constitute the Board of Directors of the Association. We have an annual audit of our business operations by a public accountant. I shall read the statement of Mr. Bisselle, the auditor. Later you will have the report of our own auditing committee.

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS FOR THE YEAR ENDED SEPTEMBER 30, 1938

Balance, October 1, 1937:

Lincoln National Bank.........................	$ 4,950.01	
Montgomery Building Association...............	81.86	$ 5,031.87

RECEIPTS

Sales:

Methods....................................	$ 7,148.35	
Journals....................................	4,944.11	
Wiley's Principles...........................	46.50	
Reprints....................................	106.75	
	$12,245.71	
Less: Discounts Allowed............. $1,426.15		
Refunds..................... 51.62	1,477.77	

Net Sales... | | 10,767.94

Other Income:

Advertisements..............................	$ 454.70	
Interest on Investments......................	392.42	
Over and Short..............................	9.97	

Total Other Income.. | | 857.09

Miscellaneous Receipts:

Federal-American Bank, Liquidating Dividend....	$ 11.08	
Returned Checks Made Good..................	58.00	
Books Ordered Through Association............	742.19	

Total Miscellaneous Receipts.................................. | | 811.27

		$17,468.17

DISBURSEMENTS

Expenses:

Salaries....................................	$ 1,200.00	
Postage.....................................	547.00	
Meeting and Association Expenses..............	185.90	
Stationery and Supplies.......................	81.50	
Auditing......................................	150.00	
Premiums, Employees' Bonds...................	10.00	
Safe Deposit Rental..........................	3.30	
Exchange.....................................	4.03	
Printing and Binding.........................	4,275.48	
Freight......................................	76.39	
Notary Fees.................................	10.00	
Total Expenses...		$ 6,543.60

Miscellaneous Disbursements:

Books Ordered Through Association............	$ 835.10	
Returned Checks.............................	48.75	
3 U. S. Treasury Bonds.......................	3,065.63	
U. S. Savings Bonds ($5,500 at Maturity)........	4,125.00	
Total Miscellaneous Disbursements.............................		8,074.48

Balance, September 30, 1938:

Lincoln National Bank.........................	$ 2,764.55	
Montgomery Building Association...............	85.54	2,850.09
		$17,468.17

BALANCE SHEET AS AT SEPTEMBER 30, 1938

ASSETS

Current Assets:

Cash in Banks:			
Lincoln National Bank...........	$2,764.55		
Montgomery Building Association.	85.54	$ 2,850.09	
Accounts Receivable................	$3,499.46		
Less: Reserve for Doubtful Accounts	120.80	3,378.66	
Inventories..................................		4,801.36	
Total Current Assets...			$11,030.11

Investments:

Home Owners Loan Corporation Bonds..........	$ 1,000.00	
Federal Land Bank Bonds......................	6,000.00	
United States Treasury Bonds.................	7,000.00	
United States Savings Bonds..................	4,125.00	
Total Investments (Par Value).................................		18,125.00

Cash in Closed Banks:

Federal-American Bank & Trust Company	$	25.85	
Commercial National Bank		96.34	122.19

Furniture and Fixtures 97.26

Total Assets $29,374.56

SURPLUS

Balance, October 1, 1937 .... $25,873.23

Add: Net Profit, for the Year 3,559.33

$29,432.56

Less: Adjustment for Returned Checks Entered

Twice 58.00

Balance, September 30, 1938 ....$29,374.56

Approved.

W. W. SKINNER

H. R. Kraybill: I am sure it is a satisfaction to all the members of the Association to see the excellent financial condition of the Association, and I am sure, Dr. Skinner, that all of the members appreciate very much the excellent service that you have given to the Association. As there was a time when we were not in as good a financial condition as we are now, we can appreciate this service a great deal.

REPORT OF COMMITTEE ON FELLOWSHIPS

To perpetuate the memory of Doctor Harvey W. Wiley, Chief of the Bureau of Chemistry, 1884 to 1912, and for 23 years Secretary of the Association of Official Agricultural Chemists, it is proposed that this Association establish

THE WILEY MEMORIAL AWARDS

These awards have two objectives (a) to stimulate undergraduates to enter the field of chemistry in its relation to agriculture, and (b) to encourage research upon problems within the scope of the Association's activities. The awards are to be made in accord with the following outline.

Procedure for Selection of Designates

Undergraduate Awards.—Respective awards of $300, $200, and $100 each will be made annually for the three best presentations, either as theses, compilations, or resumes that may be offered upon any one of the subjects dealt with in the several chapters of the Association's *Methods of Analysis.* Any member of the current senior class in any accredited college or university of North America shall be eligible to compete for the award on the following basis.

The faculty of the Department of Agricultural Chemistry or chemists of any accredited college or any university in North America shall be privileged to submit one competitive paper and to nominate its author as a candidate for the award.

The nominee so selected shall be from among those seniors in that institution who shall have presented competitive papers. The authors of the first, second, and third best contributions from the several institutions shall receive the first, second, and third awards, and each of these competitive papers shall become the property of the A.O.A.C. The contributions must be in the hands of the Secretary of the A.O.A.C. on or before August 1 of the current year. Announcement of the awards will be made at the succeeding annual meeting and in the columns of appropriate chemical and other scientific journals.

Suggested Method for Selection of Designates

Decision as to the Wiley Memorial Awards for undergraduate competitive papers and selection of the designate shall be made through a standing committee of five (5) to be designated by the President of the Association from its membership for that specific purpose, and a majority of that committee shall be requisite for a decision. Should such a majority vote of the committee not be accorded a competitive paper, the paper or the nominee receiving more than one vote within the standing committee shall be cited to a committee of three appointed by the President of the Association and the decision of this special committee shall prevail. Except for the foregoing qualification, the award of the standing committee shall be final. The right to publication of the thesis presented by the recipient of the award shall be reserved to the Editorial Board of the Association of Official Agricultural Chemists.

In the establishment of an appropriate and permanent memorial to Doctor Wiley two objectives are covered in the foregoing outline. The three awards to undergraduates are adequate to encourage participation by students in chemistry and such participation would undoubtedly stimulate an interest in chemical problems that relate to agriculture. A compilation of the names of the designates proposed by the several institutions would be useful to those who contemplate employing recent graduates. Moreover, the contributions submitted and brought together from so many different sources would undoubtedly be useful to referees and to collaborators in carrying out the work of the Association.

The total expense per annum to the A.O.A.C. would be $600.

W. H. MacIntire, Chairman
W. B. White
E. M. Bailey

No report was given by the Committee to Cooperate with other Committees on Food Definitions, as no meeting of this committee had been held. (See following notice of the appointment of a new Food Standards Committee by Secretary of Agriculture Wallace.)

FOOD STANDARDS COMMITTEE

Secretary of Agriculture Henry A. Wallace recently approved the recommendation of the Food and Drug Administration for the appointment in that organization of a Food Standards Committee, which will function under the terms of the Food, Drug, and Cosmetic Act of June 25, 1938.

It is the intention of the Food and Drug Administration to perpetuate the food standards committee that has functioned in promulgating administrative standards for the last 25 years, reports W. G. Campbell, Chief. This committee has consisted of representatives from the Association of Official Agricultural Chemists; the Association of Dairy, Food and Drug Officials of the United States; and the U. S. Department of Agriculture.

Members of the new committee are:

For the Association of Official Agricultural Chemists: C. D. Howard, Director and Chief, Division of Chemistry and Sanitation, State Board of Health, Concord, N. H.; Guy G. Frary, State Chemist, Vermillion, S. D.

For the Association of Dairy, Food and Drug Officials of the United States: J. J. Taylor, State Chemist, Department of Agriculture, Tallahassee, Fla.; Mrs. F. C. Dugan, Director, Bureau of Foods, Drugs and Hotels, State Board of Health, Louisville, Ky.

For the Food and Drug Administration: W. B. White, Chief, Food Division; W. S. Frisbie, Chief, Division of State Cooperation.

Mr. Joseph Callaway has been appointed Secretary of the committee.

The duties of the committee will be to formulate definitions and standards both of identity and of quality, including fill of container, after consideration of all of the data—analytical and inspectional—deemed essential to support them. The committee will meet in the Food and Drug Administration at Washington at intervals to review the data which have been assembled, draft tentative standards, and then upon the conclusion of public hearings, required by the act, make its final recommendations. Under the new act these definitions and standards when promulgated by the Secretary will have the full force and effect of law.

The first committee to consider food standards was one appointed by the Association of Official Agricultural Chemists in 1897. This committee was originally headed by Dr. Harvey W. Wiley. By virtue of the Congressional appropriation act of June 3, 1902, the Department of Agriculture was authorized to investigate food standards and the Association of Official Agricultural Chemists' committee was named for this purpose. This committee was supplemented in 1905 by the appointment of a representative of the Association of Dairy, Food and Drug Officials. The tripartite form of the committee was established as the result of a general conference between State and Federal food and drug officials held in November, 1913.

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REPORT OF AUDITING COMMITTEE

The public accountant's audit of the books of the Association of Official Agricultural Chemists, Inc., as of September 30, 1938, was examined by the Committee and found to be correct. Verification was also made of the bonds on deposit.

GORDON HART

Approved.   F. HILLIG

REPORT OF THE COMMITTEE ON NECROLOGY

Since our previous meeting the Association has lost by death four distinguished members, all of |them closely identified for many years with agricultural chemical work in the United States. Two of these departed colleagues, Richard Newman Brackett and John Phillips Street, were former Presidents of our Association, and their work was of such an outstanding character that their passing deserves more than a passing notice.

The life and work of Doctor Brackett have been pictured so sympathetically by his friend, Dr. W. H. MacIntire, in the last May number of our *Journal* that there is nothing which can be added to his sketch in the way of eulogy or appreciation. It was exactly 38 years ago, on November 16, 1900, that Richard Brackett attended for the first time a meeting of our Association. This was immediately after his appointment as assistant chemist at the Agricultural Experiment Station of Clemson College, S. C. His active participation in the work of the Association did not begin, however, until 1910, when he became Acting Professor of Chemistry and Acting Chief Chemist of the Fertilizer Division at Clemson. From that date until his retirement in 1932 he was a constant attendant at our meetings and an ardent promoter of all its varied activities. His inspiring presence at our annual gatherings and his beautiful spirit of friendly cooperation are fresh in the memories of nearly all of us.

Less known to the present generation of agricultural chemists but no less influential in its day was the work of John P. Street, who from 1890 until his retirement from agricultural chemical work in 1918 rarely missed a meeting of this Association. Those of us whose memories go back 30 and 40 years will recall the dynamic force which Street injected into the work of our meetings as reporter and referee on methods of fertilizer analyses, as member and chairman of numerous committees, as president of the Association at the memorable Jamestown Exposition Meeting in 1907, as member of the boards of editors of our *Journal* and of the revision of our *Methods of Analysis* and in numerous other ways. He served as Major in the World War, and upon his discharge in 1919 severed his connection as Chemist of the Connecticut Experiment Station to become Director of Inspection of the National Canners Association in Indiana. With this change in vocation Street's work as agricultural chemist and as member of this Association came to an abrupt end. It was a source of regret that thereafter we saw him no more at our annual meetings. During the last 17 years of his life up to the time of his death he was Secretary of the Association of New York State Canners, Inc. He passed away on September 22, 1938, at the age of 69 years. His outstanding services to our Association during 28 eventful years of its history will be commemorated by a more comprehensive sketch in a future issue of *The Journal*.

Another member of our Association whose presence will be greatly missed at our annual meetings is Victor King Chesnut who died on August 29, 1938, at the age of 71 years. He was born at Nevada City, Calif., June

28, 1867, and graduated from the University of California in 1890. After a brief period as instructor in chemistry at the University of California he served from 1894 to 1904 as botanist of the U. S. Department of Agriculture in charge of poisonous plant investigations. From 1904 to 1907 he was chemist of the Montana Experiment Station and from 1907 to 1927 a member of the chemical staff of the U. S. Bureau of Chemistry. From 1927 until his retirement in 1933 he was associated with the work of the U. S. Food and Drug Administration. Mr. Chesnut is best known for his investigations on poisonous plants and for his collaborations in plant chemistry with the late Dr. Frederick B. Power, with whom he was co-author of numerous publications on the volatile constituents of apples, peaches, grapes and the cotton plant and on other phytochemical subjects. The official Power-Chesnut method of our Association for determining caffeine was one of the outstanding results of this cooperation. Mr. Chesnut from 1895 until his recent death was a frequent attendant at the meetings of our Association, in the work of which he took a deep interest. His genial nature won him a host of friends in both chemical and botanical circles.

The fourth death among our membership which your committee has to record is that of George Walter Cavanaugh. He was born at Watertown, N. Y., on February 4, 1870 and died on July 2, 1938, at the age of 68 years. He graduated from Cornell in 1896 and immediately thereafter became assistant in agricultural chemistry at the Cornell University Experiment Station. He was promoted to the professorship of agricultural chemistry at Cornell in 1905 and held this position until his retirement in 1937 when the chair which he occupied was abolished. Professor Cavanaugh began attendance at our meetings in 1902, at which time he presented a report as referee on dairy products. He was also appointed Referee on Dairy Products and Associate Referee on the Adulteration of Vegetables for the 1903 meeting of the Association. Although not an attendant of our conventions during the past 20 years, Professor Cavanaugh had a wide acquaintance among agricultural chemists of the United States who will long cherish the memory of his kindly amiable personality.

I move you, Mr. President, that this report be published in the Proceedings of our Association and also that we rise for a moment as a token of respect to the memory of these departed members.

<div style="text-align: right">C. A. BROWNE
H. C. LYTHGOE</div>

Approved. _____

REPORT OF NOMINATING COMMITTEE

The Committee on Nominations wishes to present the following candidates:

President: W. S. Frisbie, U. S. Food and Drug Administration, Washington, D. C.

Vice-President: L. B. Broughton, College Park, Md.

Secretary-Treasurer: W. W. Skinner, U. S. Bureau of Chemistry and Soils, Washington, D. C.

Additional Members of the Executive Committee: J. W. Sale, Washington, D. C.; G. G. Frary, Vermillion, S. Dak.; J. O. Clarke, Chicago, Ill.; *Post-Officio:* H. R. Kraybill, Lafayette, Ind.

<div align="right">

H. H. HANSON
H. A. LEPPER
W. H. MacINTIRE

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A unanimous vote was cast for the officers nominated.

REPORT OF COMMITTEE ON RESOLUTIONS

Whereas, the Fifty-Fourth Annual Meeting of the Association of Official Agricultural Chemists is being concluded; and

Whereas, an unusually large attendance has profited by a comprehensive and instructive program; be it

Resolved, that we express to our president, Dr. H. R. Kraybill, our appreciation of his excellent presidential address and his able and courteous direction of our proceedings as our presiding officer.

Resolved, that we express to Dr. L. M. Tolman, our appreciation of his masterly Wiley Memorial Address, "The History and Development of Food Inspection in the United States."

Resolved, that we extend our thanks to those members who have assisted the president by presiding over our several sectional meetings and to all referees and associate referees.

Resolved, that we extend our thanks to our Secretary, Dr. Skinner, to Miss Lapp, to Mr. Frisbie, and to all their associate workers, for the careful thought and attention given by them to insure the interest of members and guests and the success of the meeting.

Resolved, that, through our Secretary, we extend our thanks to the management of the Raleigh Hotel for their cooperation and courtesy.

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W. CATESBY JONES
GEORGE H. MARSH

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Approved.

CONTRIBUTED PAPERS

DETERMINATION OF VOLATILE FATTY ACIDS AS AN APPROACH TO THE EVALUATION OF SPOIL-AGE IN CANNED HERRING ROE

By FRED HILLIG (Food Division,* Food and Drug Administration, Department of Agriculture, Washington, D. C.)

In previous communications there was described a modification of the Dyer method (itself a modification of the well-known Duclaux procedure)
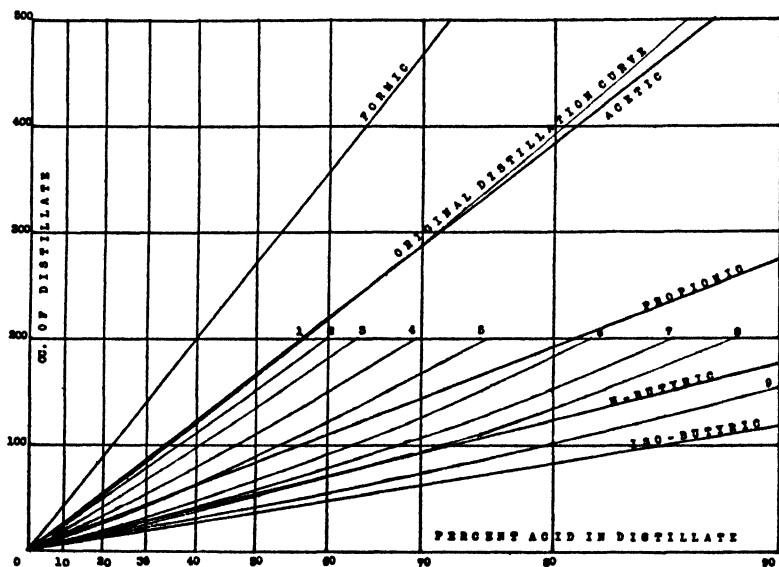
FIG. 1.—FRACTIONATION OF VOLATILE ACIDS FROM HERRING ROE, CODE 4

for the determination of volatile fatty acids[1] and the application of this method to the determination of volatile fatty acids in canned salmon and tuna fish[2] as an approach to the problem of evaluating spoilage.

The work has now been extended to cover the determination of volatile fatty acids in canned herring roe, and the purpose of this paper is to present the facts developed.

As in the case of salmon and tuna fish, it was found that canned herring roe prepared from the freshest possible raw material contains small quantities of volatile fatty acids. When spoilage begins the quantity of volatile fatty acids is increased, and as decomposition progresses there is likewise a progressive increase in these acids.

An attempt was then made to identify the individual acids comprising the acid mixture. A quantity of volatile fatty acids was obtained by steam

distilling a clarified water extract of the roe (see under "Method"). A distillation curve (Curve 1, Figure 1) was prepared, and the acids were fractionated according to the procedure given in the previous report.[2]

TABLE 1.—*Analysis of canned herring roe*

CAN NO.	CODE 1		CODE 2		CODE 3		CODE 4	
	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER
	mg./100 g.	*cc. 0.01 N/ 100 g.*	*mg./100 g.*	*cc. 0.01 N/ 100 g.*	*mg./100 g.*	*cc. 0.01 N/ 100 g.*	*mg./100 g.*	*cc. 0.01 N/ 100 g.*
1	Trace	6.1	0.97	15.2	2.22	25.1	3.96	72.9
		6.0	1.01	14.8	2.11	24.6	3.99	73.1
2	Trace	7.1	1.40	16.3	2.22	25.1	4.09	75.2
		7.1	1.30	16.0	2.22	25.1	4.09	74.8
3	Trace	7.1	1.40	16.5	2.21	25.3	3.96	75.7
		7.3	1.30	16.5	2.11	25.0	3.99	74.6
4	Trace	7.4	1.33	16.7	2.34	29.1	4.25	78.5
		7.5	1.20	16.6	2.44	29.1	4.12	78.4
5	Trace	7.6	1.40	17.3	2.50	29.1	4.03	79.1
		7.5	1.40	17.1	2.44	29.4	4.03	80.6
6	Trace	8.1	1.27	17.8	2.50	29.9	5.03	83.9
		8.0	1.20	17.5	2.47	29.8	4.77	84.6
7	Trace	8.3	1.46	18.3	2.37	31.6	4.74	84.9
		8.3	1.46	18.0	2.37	30.8	4.51	84.1
8	Trace	8.2	1.62	20.2	3.25	38.4	4.64	87.6
		8.4	1.59	20.1	2.89	37.6	4.61	87.3
9	Trace	8.6	1.75	20.1	3.41	40.4	5.03	87.9
		8.4	1.69	20.3	3.47	41.0	4.84	87.5
10	Trace	8.6	1.69	20.1	3.92	44.2	5.75	95.7
		8.7	1.75	20.3	3.92	44.4	5.88	95.3
Av.		7.7	1.41	17.8	2.66	31.8	4.51	82.1
Min.		6.0	0.97	14.8	2.11	24.6	3.96	72.9
Max.		8.7	1.75	20.3	3.92	44.4	5.88	95.7

Curve 1 starts below the acetic acid line, intersects it, and approaches the formic acid line. Since the curve intersects the acetic acid line, formic acid and one or more acids higher in the series than acetic acid are indicated. The angle at which the curve crosses the acetic acid line indicates the presence of this acid. Since fractionation curves 8 and 9 fall below the curve for N-butyric acid, they show that the highest detectable member of the series of acids present is iso-butyric acid.

METHOD

Volatile acid number.—Pass the entire contents of a can of roe through a meat chopper three times and thoroughly mix the material after each grinding. Weigh 50 grams of this material into a 250 cc. beaker, stir it to a uniform suspension with 100 cc. of water, and then quantitatively transfer it to a 250 cc. volumetric flask. Add 2 cc. of 2 N H_2SO_4, dilute to 250 cc., *shake vigorously*, allow to stand 5 minutes, and filter through a folded paper. Transfer 150 cc. of the filtrate to the distillation flask, and proceed as previously directed.[2]

Formic acid number.—Proceed as previously directed.[3]

The methods were applied to four experimental packs of herring roe, with the results shown in Table 1. Code 1 was prepared from raw material of unquestionable freshness, while the succeeding codes represent progressive stages of spoilage up to and including badly tainted roe.

The results require no comments.

The procedure was then applied to seven commercial samples of herring roe, with the results given in Table 2.

TABLE 2.—*Analysis of commercial canned herring roe*

SAMPLE NO.	FORMIC ACID NUMBER	VOLATILE ACID NUMBER
	mg./100 g.	*cc. 0.01 N/100 g.*
1	2.24	24.9
2	Trace	8.9
3	Trace	13.0
4	Trace	10.7
5	Trace	12.2
6	Trace	12.8
7	Trace	11.1

SUMMARY

A method for the evaluation of spoilage in canned herring roe is presented. The procedure is simple and yields accurate, consistent results.

ESTIMATION OF CALOMEL IN COMPOUND CATHARTIC PILLS

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The National Formulary VI does not give a method of assay for calomel in Compound Cathartic Pills, but the iodine method is provided for tablets of Calomel and Calomel and Soda. The basically similar A.O.A.C method for Calomel in Tablets (*Methods of Analysis*, 1935, p. 595, 151) was tried on Compound Cathartic and products of the same type, i.e., calomel

in mixture with plant drugs, with disappointing results. They were usually high and very inconsistent. The calomel could not be satisfactorily separated from the organic matter, which consumed some of the iodine. Even after thorough washing with alcohol, ether, acidulated water, etc., pills or tablets of Compound Cathartic gave results from 1.7 to 41.3 per cent higher than those obtained by a modified gravimetric sulfide procedure.

The sulfide procedure follows:

Weigh a sufficient number of pills or tablets to give a representative average weight, then reduce to a medium fine powder. Transfer a portion of the well-mixed sample, calculated to yield 100–300 mg. of Hg, to a comparatively thick, well-packed mat of asbestos in a Caldwell crucible. Wash the sample with small successive portions of warm alcohol until the filtrate becomes colorless. Repeat the washings with ether, dilute acetic acid, alcohol, and ether. (Cautiously stir the residue in the crucible during each washing to materially shorten the number of washings necessary to obtain colorless filtrates.) Air-dry the residue by a few minutes' suction after the ether washing.

Transfer the insoluble residue and the asbestos mat to a 250 cc. centrifuge bottle and add from a pipet 200 cc. of saturated bromine water. Stopper the bottle and shake vigorously for 10–15 minutes, let stand for 5–10 minutes, then again shake for 10–15 minutes. Centrifuge it to throw down the insoluble matter, and thus obtain a supernatant solution with comparatively small amounts of organic matter.

Pipet an aliquot of the supernatant solution, calculated to yield about 100 mg. of Hg, into a 500 cc. Erlenmeyer flask, dilute to about 200 cc., and add 5–10 cc. of concentrated H_2SO_4. Boil the solution for 5–10 minutes to expel the excess bromine. Place the flask on a steam bath and add successive small portions of powdered $KMnO_4$ until the bright pink color of the permanganate persists for 5–10 minutes. Clear the solution of precipitated oxides of Mn and excess permanganate by adding, dropwise, dilute H_2O_2. Dissipate any excess peroxide by adding very dilute permanganate solution or boiling for 2–3 minutes. Filter the solution, and wash the filter well with about 100 cc. of water in small portions, collecting the washings with the filtrate. Cool the solution to room temperature, then saturate with a slow stream of H_2S. Filter, wash, dry, and weigh the HgS as usual.

Weight of the HgS $\times 1.0146 =$ HgCl.

The following modification of the procedure has given equally concordant results:

Wash a portion of the powdered sample calculated to yield about 100 mg. of Hg as directed above—then, instead of placing the insoluble residue and asbestos mat in a centrifuge bottle, transfer directly to an Erlenmeyer flask with about 200 cc. of water. Add 5–10 cc. of concentrated H_2SO_4, oxidize with powdered permanganate, and clear with peroxide. Next add a few drops of pure bromine to impart a definite brown color to the solution and carefully shake the flask for a few minutes to insure the oxidation of the Hg. Filter off the insoluble matter through a Gooch, wash the filter well with water, then boil the combined filtrate and washings to drive off the bromine. Cool the solution, saturate with H_2S, and finish the determination as usual.

The recoveries obtained from mixtures of calomel and starch are given in Table 1.

TABLE 1.—Recoveries from mixtures of calomel and starch

SAMPLE	CALOMEL ADDED	CALOMEL OBTAINED	RECOVERY
	gram	gram	per cent
1	0.1340	0.1319	98.4
2	0.1227	0.1224	99.8
3	0.2095	0.2110	100.7
4	0.1332	0.1311	98.4
5	0.0920	0.0918	99.8
6	0.1005	0.0989	98.4
7	0.1014	0.0982	96.9
8	0.1545	0.1540	99.7
9	0.1551	0.1553	100.3
10	0.2432	0.2418	99.4

Table 2 shows the results obtained from mixtures of calomel and plant drugs as required for Compound Cathartic Pills.

TABLE 2.—Recoveries from mixtures of calomel and plant drugs

SAMPLE	CALOMEL ADDED	CALOMEL OBTAINED	RECOVERY
	gram	gram	per cent
11	0.1531	0.1544	100.8
12	0.1512	0.1535	101.5
13	0.1513	0.1324	100.7
14	0.1098	0.1115	101.5
15	0.1308	0.1308	100.0
16	0.1238	0.1245	102.1

Lastly, commercial samples of Compound Cathartic Pills were assayed by these sulfide modifications. Known amounts of calomel were then added, and from the difference of the two results the recoveries were calculated (Table 3).

TABLE 3.—Recoveries when known amounts of calomel were added

SAMPLE	CALOMEL ADDED	CALOMEL OBTAINED	RECOVERY
	gram	gram	per cent
17	0.2381	0.2359	99.1
18	0.2003	0.2035	101.6
19	0.1712	0.1709	99.8
20	0.1410	0.1388	98.4
21	0.2246	0.2220	98.8
22	0.1383	0.1383	100.0
23	0.1585	0.1560	98.4
24	0.2000	0.1992	99.6
25	0.2000	0.2015	100.7

From the experience in the above work, it seems reasonable to conclude (1) that the official iodine method for the determination of calomel in tablets—presumably for other than the official tablets of Calomel or Calomel and Soda, should be deleted, or its use restricted to tablets of comparatively simple composition; (2) that the modified procedures presented here for the gravimetric estimation of mercury in mixtures of calomel and plant drugs by the sulfide precipitation method give acceptable results.

DETERMINATION OF ARSENIC

By A. K. KLEIN and F. A. VORHES, JR. (U. S. Food and
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The Gutzeit method,[1] modified by a "solvent" procedure[2] for preparation of the sample, thereby avoiding "wet ashing," is extensively used in determining spray residue on fresh fruit. The method is convenient and rapid, and in the hands of the experienced analyst gives results sufficiently accurate for most purposes. More accurate methods are available, but they generally demand complete destruction of organic matter by wet ashing and isolation of the arsenic by distillation, and so are necessarily somewhat time-consuming. It is the purpose of this paper to present a method that appears to be more accurate than the Gutzeit, but which may be used on samples prepared by the solvent procedure as well as by wet ashing. As described, the proposed method was applied to a range of arsenic content midway between and overlapping the ranges covered by the Gutzeit and bromate[3] methods, but suggestions are offered for adapting it to either higher or lower ranges.

Deniges' method,[4] as improved by Zinzadze,[5] gives excellent results provided the arsenic is first separated from interferences. This may be accomplished by destroying organic matter and distilling the arsenic as the trichloride.[6,7] The work of Tarugi and Sorbini,[8] however, suggests that the isolation may be effected more conveniently by the use of immiscible solvents. They reported the quantitative precipitation of arsenic xanthate from an acidified aqueous medium and described the isolation of minute amounts of the precipitate by extraction with organic solvents. They used this means of separation in connection with a qualitative test for arsenic. They also took advantage of the solubility of arsenic xanthate in chloroform to purify their yield in the gravimetric estimation of larger

[1] *Methods of Analysis*, A.O.A.C., 1935, 370.
[2] *Ibid.*, 391.
[3] *Ibid.*, 373.
[4] *Compt. rend.*, 171, 802 (1920).
[5] *Ind. Eng. Chem. Anal. Ed.*, 7, 227 (1935).
[6] Deemer and Schricker, *This Journal*, 16, 230 (1933).
[7] Cf. Burkard and Wullhorst, *Z. Untersuch. Lebensm.*, 70, 308 (1935): abs. *Analyst*, 61, 198 (1936).
[8] *Boll. Chim. Farm.*, 51, 361 (1912): Abs. *Chem. Zentr.*, 1912, II, 1399.

amounts of arsenic but apparently did not adapt the direct extraction to a quantitative method for small amounts.

The following detailed procedure is based on the findings of Tarugi and Sorbini and the method of Zinzadze. The main steps are as follows: (1) Preparation of the sample by solvent procedure or wet ashing. (2) Reduction of arsenic to trivalent form with KI and acid. (3) Simultaneous formation and extraction of arsenic xanthate by use of a solution of sodium xanthate in CCl_4 and alcohol. (4) Removal of CCl_4-soluble interferences (if present) by washing the extract through concentrated HCl containing $SnCl_2$. (5) Elimination of CCl_4 by volatilization. (6) Oxidation of arsenic to pentavalent form with bromine. (7) Development of the molybdenum-blue color with Zinzadze's reagent. (8) Estimation of the color by means of the neutral wedge photometer.

METHOD

PREPARATION OF SAMPLE

Solvent procedure.—Applicable to apples, pears, bell peppers, green tomatoes, and similar firm fruit. Follow **XXIX**, 30, p. 391, *Methods of Analysis, A.O.A.C.*, 1935. Use HCl for rinse and acidification.

Wet ashing.—Generally applicable to all types of samples. Follow **XXIX**, 3(a-b-c-d), pp. 371-2, *Methods of Analysis, A.O.A.C.*, 1935, using 20 cc. of H_2SO_4. The sample should contain not more than 0.8 mg. of As_2O_3.

REAGENTS

(1) *Sulfuric acid.*—Reagent quality, arsenic-free.

(2) *Potassium iodide.*—10% W/V solution of the C.P. salt.

(3) *Sodium thiosulfate.*—Approximately 0.1 N solution.

(4) *Hydrochloric acid.*—Reagent quality, arsenic-free.

(5) *Stannous chloride.*—40% W/V solution in concentrated HCl.

(6) *Bromine water.*—Saturated.

(7) *Carbon tetrachloride.*—Reagent quality.

(8) *Zinzadze's reagent.*—Follow exactly the directions given by Zinzadze—loc. cit.

Solution I.—Take, in a 3 liter Erlenmeyer flask, 1010 cc. of 25 N H_2SO_4; add acid molybdic (anhydride) containing exactly 40.11 grams of MoO_3; boil very gently, with occasional shaking, just until solution is complete, avoiding the evolution of white fumes; cool to room temperature; dilute with distilled water to about 998 cc. and cool again. Finally make up to exactly 1 liter with distilled water and mix well. (The solution has a bluish color.)

Solution II.—Place 500 cc. of Solution I in a 3 liter Erlenmeyer flask; add 1.78 grams of Mo powder and boil very gently (with precautions as before) for exactly 15 minutes from incipient boiling, shaking from time to time. Allow to cool to room temperature; decant the solution from the small residue that may be present into a 500 cc. volumetric flask; dilute with distilled water to about 498 cc. and cool again. Finally make up to exactly 500 cc. with distilled water and mix well. (The solution has a greenish blue color.)

Dilute a 5 cc. aliquot of Solution II to about 50 cc. with distilled water and titrate with 0.1 N $KMnO_4$. (It is necessary to use a pipet previously wet inside with water and washed down afterward with a few cc. of water in order to deliver 5 cc. of the viscous reagent accurately.)

Finally prepare the molybdenum blue reagent by mixing certain quantities of Solutions I and II, so that 5 cc. of the resulting mixture corresponds to 5 cc. of 0.1 N KMnO$_4$. (It will keep at least 4 years, and probably indefinitely, provided it is of sufficient purity and is kept free from dust, vapors, and other contamination in a glass-stoppered Pyrex bottle.)

It is suggested that the analyst refer to the original paper for specifications as to purity and allowable tolerances.

(9) *Sodium ethyl xanthate solution.*—To 10 parts of absolute ethyl alcohol (cc.) in a centrifuge bottle add 1 part (gram) of pure NaOH pellets. Warm to about 40°C. and shake vigorously in a shaking machine for about 15 minutes. Centrifuge and pour off the supernatant liquid. Ascertain the NaOH content by titration and adjust it to 7% W/V NaOH by addition of absolute alcohol. To 9 volumes of the adjusted solution, add slowly, with cooling, 1 volume of colorless carbon disulfide, mix, and filter. The solution contains approximately 22% sodium xanthate.

NOTE: This reagent is not generally available in pure (solid) form from commercial sources. Its preparation in the laboratory in dry form requires considerable time and the use of large quantities of ether. Attempts to prepare a stable solution of it by various means were without particular success. The described preparation, however, is relatively simple to make and does not decompose to an excessive extent for about 10 days—or even longer when stored in a refrigerator.

(10) *Extraction reagent.*—Mix Reagent 9 with CCl$_4$ in the proportion of 50 cc. per liter. The reagent should be made up in amounts to last 1 day only, as it decomposes appreciably within 36 hours.

(11) *Standard arsenic solution.*—Dissolve 1 gram of Bureau of Standards As$_2$O$_3$ in 25 cc. of 30% NaOH. Neutralize with dilute H$_2$SO$_4$ and dilute to 1 liter. Dilute 50 cc. of this solution to 500 cc. 1 cc. = 0.1 mg. of As$_2$O$_3$.

DETERMINATION

To samples prepared by wet ashing add 50 cc. of water, mix thoroughly, and while still hot, add 20 cc. of the KI solution. Transfer the mixture to a 250 cc. separatory funnel, using about 50 cc. of water, in portions of 10–25 cc., to rinse out the flask.

Select aliquots of samples prepared by the solvent procedure so as to contain not more than 0.8 mg. of As$_2$O$_3$, and, for reasons of mechanical manipulation, not to exceed a volume of 200 cc. Transfer the aliquot to a separatory funnel of appropriate size, add a volume of the concentrated H$_2$SO$_4$ equal to 1/5 the volume of the aliquot, and mix. While the mixture is still hot, add a volume of KI solution equal to the volume of H$_2$SO$_4$ used, and mix.

Allow the sample (prepared by either means) to stand until the funnel is no longer uncomfortably warm to the hand and then dispel the liberated I$_2$ with the Na$_2$S$_2$O$_3$ solution, mixing thoroughly and carefully avoiding an excess of more than 0.5–1.0 cc.

Regardless of aliquot size, add 25 cc. of the extraction reagent, stopper the funnel *immediately*, and shake *vigorously* for 1–2 minutes. Allow the layers to separate.

NOTE: At this point the presence of antimony in amounts greater than a few mg. is indicated by a brick red or yellow precipitate which tends to gather at the interface; the presence of tin or small amounts of copper is indicated by a deep yellow to orange color in the lower layer; a large amount of copper is indicated by a yellow precipitate suspended in the lower layer and tending to gather at the interface.

Draw off the lower layer into a second (125 cc.) separatory funnel containing 50 cc. of the concentrated HCl and 1 cc. of the SnCl$_2$ solution. Wash down the first funnel with a few cc. of CCl$_4$ and draw the washings, without shaking, into the sec-

ond funnel so that any residual extract in the stem of the first funnel is displaced with relatively pure CCl₄.

NOTE: The second funnel must be dry when the acid is placed in it for the reason that even slight dilution destroys its effectiveness. Make no effort to draw the precipitate, if any, into the second funnel as this frequently results in entrainment of some of the aqueous phase and may adversely affect the HCl–SnCl₂ wash in removal of interferences. No harm is done, however, if some of the precipitate passes through, suspended in the organic phase.

Stopper and shake the second funnel vigorously for about 2 minutes, or until the lower layer becomes a very pale, clear yellow and the upper layer is either completely clear or at the most contains only a small amount of suspended matter.

Draw off the lower layer through a plug of cotton about 1/2 inch long, packed loosely but uniformly into the stem of the funnel, into a third separatory funnel containing 50 cc. of H₂SO₄ (1+200). Shake vigorously, and draw off the lower layer, through a plug of cotton, into a 125 cc. Erlenmeyer flask marked at the 60 cc. capacity point and containing 10 cc. of water and 2 or 3 small glass beads.

Repeat the entire extraction with two additional 15 cc. portions of the extraction reagent, adding them to the first funnel and carrying them, separately, through the process, finally combining all three extracts in the Erlenmeyer.

NOTE: When tin, antimony or large amounts of copper are absent, the second separatory funnel may be eliminated; the first funnel is fitted with a cotton plug and the extracts drawn directly into the funnel containing the H₂SO₄ (1+200), shaken vigorously, and then drawn into the Erlenmeyer.

Always invert the funnels when shaking in order to avoid loss of drops of the extract retained in the stems.

Evaporate the CCl₄ on a hot plate and bring the aqueous solution to a vigorous boil for at least 1 minute, preheating the hot plate and swirling the flask a little at the start to avoid "bumping." Wash down the sides of the flask with a little water and add 20 cc. of the bromine water. Insert between flask and hot plate a piece of asbestos board of a thickness such that the solution reaches gentle boiling in 2–5 minutes, and continue boiling gently until the bromine, as judged by color, is almost dispelled. Finally, boil vigorously on the bare hot plate to remove the bromine completely.

Dilute to 60 cc.; add, by means of a pipet, exactly 10 cc. of the Zinzadze reagent (10-fold dilution), bring to a boil, and boil steadily but not vigorously for just 5.5 minutes. Cool, transfer to a 100 cc. volumetric flask, dilute to volume, and mix.

Fill a 1-inch cell with the blue solution and obtain the direct reading in the neutral wedge photometer.[1]

To the solution remaining (should be more than 50 cc.) add about 1 cc. of bromine water and warm slightly until all blue or green shades are dispelled and only the yellow color of bromine remains. (Only a small amount of bromine water is required but a substantial excess over that necessary to just bleach the blue must be present.) Obtain the photometer reading of the yellow solution (termed the "bromine blank") and subtract it from the direct photometer reading. (This correction compensates for slight turbidities which may be present.)

CALIBRATION OF PHOTOMETER

To calibrate the photometer, prepare, in duplicate or triplicate, a series of 6 or 8 standards (extending over the range 0–0.8 mg. As₂O₃), adding the required amounts of Reagent 11 to 50 cc. portions of water in separatory funnels and carry them through all steps of the determination (except that the HCl–SnCl₂ wash may be omitted).

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[1] Clifford and Wichmann, *This Journal*, 19, 130 (1936).

With a photometer equipped with a Wratten neutral gelatine wedge and a glass color filter consisting of 4.5 mm. Corning dark pyrometer red No. 241, a linear relationship is obtained between photometer reading and amount of arsenic present. The writers have used this combination in most of their work. An objection to the gelatine wedge is that it will begin to show deterioration after a year or so. The Jena all-glass neutral wedge overcomes this objection and gives a linear function with the same filter. The B. & L. Smoke C glass wedge, used widely for other determinations, is, unfortunately, not quite neutral in the deep red and will not, therefore, produce an exactly linear function. With this wedge it is necessary to utilize a large-scale plot of the standards to convert, graphically, the photometer reading of the sample to the amount of arsenic represented thereby. With the other wedges the equation, $Y = a + bX$, may be calculated by the method of least squares as follows:

$$b = \frac{\sum XY - \sum XM_y}{\sum X^2 - \sum XM_x}, \text{ and } a = M_y - bM_x,$$

where $\sum$ denotes "sum of" and M denotes "mean of."

Thus, $\sum X$ = sum of the X's; M_x = mean of X; M_y = mean of Y;
   $\sum XY$ = sum of the products of X and Y;
   $\sum X^2$ = sum of the squares of X;
   Y = mg. As_2O_3 extracted;

and X = photometer reading in mm. (corr. for bromine blank).

Exclude "blanks" (zero arsenic) from these calculations.

Having inserted in the linear equation the numerical values of a and b, calculated from the standards, obtain the result of a determination by substituting the photometer reading of the sample of $4X$ and solving for Y.

In use the equation is likely to be more accurate than the graphical methods. The calculation is somewhat time-consuming, but the standardization need be carried out only once provided the adjustment of the photometer is not changed and the wedge does not deteriorate.

DISCUSSION

In accordance with usual practice in colorimetric determinations, it has been specified that the standards be given as nearly as possible the same treatment that is given to samples. In this method, however, it makes very little difference whether the standards are subjected to extraction or whether they are developed directly in the Erlenmeyer flasks, starting with the bromine treatment. The standard arsenic solution must be exactly neutral if the latter is done. Nevertheless since there does appear to be a slight loss in the extraction (Table 1), analysts desiring the highest accuracy may find it worth the extra time to follow the conventional rule, carrying the standards through the extraction and even using the HCl-SnCl₂ wash in the event that the samples to be analyzed contain tin, copper, or antimony.

The analyst engaged in control work will find from experience that samples of the same character vary but little in their bromine blanks and will be able to select an approximately average bromine blank.

While the range of the method described has been set at 0–0.8 mg. of As_2O_3, this range may be extended by diluting the aqueous solution in the

TABLE 1.—*Photometer readings on pure solutions*

DESCRIPTION AND TREATMENT	ARSENIC PRESENT (EXPRESSED AS MG. As₂O₃)						
	0	0.1	0.2	0.35	0.55	0.65	0.8
	Photometer reading—mm.—corr. for bromine blank						
B.S. As₂O₃ soln—extracted	0.2	15.0	29.9	51.2	80.1	94.5	114.4
B.S. As₂O₃ soln—extracted	0.2	14.8	29.2	49.7	80.3	94.7	115.2
B.S. As₂O₃ soln—extracted	0.5	15.3	30.0	52.2	81.2	95.6	119.3
B.S. As₂O₃ soln—not extracted	0.6	15.0	29.8	51.7	80.6	94.4	117.9
B.S. As₂O₃ soln—not extracted	0.5	15.3	30.2	51.5	80.7	95.0	117.8
B.S. As₂O₃ soln—extracted with HCl-SnCl₂ wash	0.4	14.2	29.2	50.7	80.3	92.3	118.4
PbHAsO₄ soln—extracted	0.4	14.5	29.8	51.5	80.8	94.6	116.3
PbHAsO₄ soln—extracted with HCl-SnCl₂ wash	0.5	14.9	29.7	50.0	78.9	92.0	116.6
Theoretical reading based on equation of line calculated from extracted standards (1st 3 series) mg.As₂O₃ = 0.00691 mm.–0.0047	0.7	15.1	29.6	51.3	80.3	94.7	116.4

Erlenmeyer flask, before addition of Zinzadze's reagent, to twice or three times the specified 60 cc., adding two or three times the specified 10 cc. of reagent, and diluting finally to 200 or 300 cc. In an analogous manner small amounts of arsenic may probably be determined to advantage by restricting the final volume to 50 cc. or even 25 cc., also restricting the amount of reagent and the volume before addition of reagent in similar proportion.

INTERFERENCES

Zinzadze mentions iron, nitrate, and phosphate as interferences in the development of the color by means of his reagent. As none of these ions are soluble in the extraction reagent they do not constitute interferences in the described procedure. Nitrate in large amount tends to prevent the reduction of arsenate to arsenite prior to extraction but its effect is nil when it is present in the amounts normal to food products. The interference of tin and antimony is due to formation of insoluble oxycompounds of these metals during the bromine treatment and consequent heavy turbidities which interfere with proper evaluation of the color. Tin and antimony xanthates are soluble in concentrated hydrochloric acid and are decomposed to the corresponding metallic chlorides, whereas arsenic xanthate is unaffected and remains in the carbon tetrachloride layer. If hydrochloric acid alone is used to remove tin and antimony from the extract,

another interference is introduced. When copper is present under these conditions iodine almost invariably appears in the second funnel and oxidizes the arsenic xanthate, rendering the arsenic insoluble in carbon tetrachloride. Apparently cuprous iodide is formed in the first funnel and is carried, suspended in the extract, into contact with the concentrated hydrochloric acid in the second funnel. To prevent formation of iodine, stannous chloride is added to the hydrochloric acid.

It is obvious that oxidizing agents must be removed or reduced before the determination is attempted. Also, the presence of substances forming heavy precipitates in the presence of sulfate or xanthate may be expected to cause mechanical difficulties in the extraction. However, the usual components of foods, or likely contaminants thereof, in the amounts ordinarily present, have not been found to introduce more than minor mechanical difficulties. These include the alkalis, alkaline earths, aluminum, manganese, zinc, lead, mercury, cadmium, and bismuth.

The addition of thiosulfate must be restricted fairly closely to the amount required to dispel the iodine. This amount is converted to tetrathionate but any excess thiosulfate results in formation of colloidal sulfur. It was observed that when a large excess of sodium thiosulfate was used, low results were invariably obtained and also that the acidity of the final solution was increased in rough ratio to the excess of sodium thiosulfate added. Apparently colloidal sulfur dissolves to some extent in the extraction reagent and passes through to the bromine treatment where it is oxidized to form sulfuric acid.

RECOVERY EXPERIMENTS

Table 1 gives photometer readings of standards developed under varying conditions and also readings on known amounts of lead arsenate solution. Theoretical photometer readings, based on the linear equation calculated from standards developed as prescribed above, have been included. A comparison shows that there is only a negligible loss of arsenic in either the extraction or the hydrochloric acid-stannous chloride wash.

Table 2 gives the results of recovery experiments conducted under varying conditions in regard to type of sample, method of preparation, interference, and range of arsenic content.

The standard deviation calculated from the 60 results would indicate that the method is capable of an accuracy of ± 0.02 mg. (3×0.0068) in more than 99 out of 100 instances. This is equivalent to an accuracy of 0.001 gr./lb. provided a sample of, or aliquot representing, 140 grams or more is extracted.

COLLABORATIVE WORK

Three series of samples were sent to collaborators. The draft of the procedure accompanying the first series was incomplete or faulty in several

TABLE 2.—*Recoveries in presence of interferences*

(Expressed as mg. of As₂O₃ calculated from equation based on extracted standards and corrected for reagent blanks)

DESCRIPTION	INTERFERENCES—MG.							HCl-SnCl₂ WASH	ARSENIC ADDED AS PbHAsO₄—EXPRESSED AS MG. As₂O₃					
	Sb	Cu	Sn	SO₂	Fe	NO₃	P₂O₅		0.10	0.20	0.35	0.55	0.65	0.80
PbHAsO₄ solution	10	10	50					Yes	0.100	0.201	0.349	0.550	0.652	0.803
Sugar—wet ashed	10	10	50					Yes	0.094	0.194	0.346	0.550	0.651	0.802
140 grams tomatoes—wet ashed	10	10	50					Yes	0.101	0.200	0.352	0.549	0.646	0.783
Tomatoes—solvent prep'n	10	10	50					Yes	0.103	0.207	0.347	0.550	0.649	0.800
Sugar—wet ashed	10	10	50	25	50	50	250	Yes	0.103	0.200	0.349	0.546	0.643	0.786
140 grams tomatoes—wet ashed	10	10	50	25	50	50	250	Yes	0.093	0.190	0.351	0.540	0.637	0.799
Apples—solvent prep'n	10	10	50	25	50	50	250	Yes	0.100	0.201	0.345	0.537	0.642	0.783
Sugar—wet ashed				25	50	50	250	No	0.098	0.198	0.356	0.554	0.654	0.799
140 grams apples—wet ashed				25	50	50	250	No	0.097	0.193	0.348	0.550	0.647	0.796
Tomatoes—solvent prep'n				25	50	50	250	No	0.100	0.198	0.353	0.541	0.651	0.802
Standard deviation—10 results in each range									0.0034	0.0049	0.0053	0.0062	0.0057	0.0090

Standard deviation—60 results—0.0068.

respects. Primarily for this reason the results on this group were generally unsatisfactory.

After further investigation of interferences and technic, a second series was issued with a rewritten procedure, which was essentially the same as described above with one important exception—the extraction reagent contained only half the xanthate now prescribed. This concentration generally proved insufficient. The reason may be visualized somewhat as follows: When the extraction reagent is added to the strongly acid solution in the first funnel, the xanthate is disposed of in three ways: (1) converted to xanthic acid which decomposes rapidly; (2) converted to xanthates of metals such as tin, copper, and antimony (if present); and (3) forms arsenic xanthate. If the funnel is not shaken immediately after addition of the extraction reagent, reaction (1) predominates. If, at the same time, the sample contains relatively large amounts of "interferences," the residual xanthate is, by mass action, largely disposed of by reaction (2). Under these conditions, unless the reagent contains an amount of xanthate greatly in excess of that required for the arsenic alone, a quantitative extraction of arsenic is prevented.

While all analysts collaborating on the second series reported quantitative results on samples that contained only small amounts of interferences, the majority obtained low results where a relatively large amount of copper, tin, and antimony was present.

All the results of Table 2 had been obtained (by Klein) with an approximately 0.5 per cent xanthate extraction reagent, and on the basis of these results that concentration had been deemed sufficient. However, it is probable that the analyst in this instance had, through repetition, developed a technic that minimized the effect of reaction (1).

A third series of collaborative samples, identical with the second series except as to arsenic content, was first submitted to collaborators Gerritz, Bois, and McRoberts, who analyzed them under verbal directions, using the stronger extraction reagent and observing precautions as described herein. Quantitative results having been obtained, the series was then submitted to the other collaborators together with the written instructions as given above. These results are reported in Table 3.

The method has been used in this laboratory in the analysis of 100 or more routine samples of a wide variety. No difficulties of a mechanical nature were encountered. From observation and experience thus obtained it is believed that food samples ordinarily handled will contain considerable less so-called interferences than were present in the collaborative samples.

ACKNOWLEDGMENTS

TABLE 3.—*Collaborative results*

| Sample No. 1—Solvent preparation; 1400 gm. apples (or tomatoes) treated; solution made to 500 cc. after addition of 2.72 mg. As_2O_3 as $PbHAsO_4$; determination made on $100/500 \times 50/110$ acidified aliquot to which was added 5 cc. of solution "A"—see below | Sample No. 2—Wet-ashing preparation; 1 cube of lump sugar wet-ashed with 25 cc. of a solution of $PbHAsO_4$ containing .500 mg. As_2O_3 per 25 cc. and 10 cc. of solution "A"—see below |

COLLABORATORS*	CORRECT RECOVERY		CORRECT RECOVERY
	0.2923 mg. As_2O_3 or 0.0161 gr./lb.		0.590 mg. As_2O_3
	As_2O_3 Reported		As_2O_3 Reported
	mg.	*gr./lb.*	*mg.*
H. Bois (a)	0.275	0.0151	0.578
L. H. McRoberts (a)	0.298	0.0164	0.587
H. W. Gerritz (a)	0.287	0.0158	0.596
L. A. Salinger (a)	0.285	0.0157	0.604
P. A. Mills (b)	0.295	0.0162	0.563
H. W. Conroy (c)	0.295	0.0162	0.592
P. A. Clifford (d)	0.294	0.0162	0.578
W. Stoneburner †	0.290	0.0160	0.510

Solution "A" contained (per cc.)

mg.			*mg.*		
0.009	As_2O_3	as $PbHAsO_4$	0.4	Sb	as $K(SbO)C_4H_4O_6$
1.0	Cu	as $CuSO_4$	5.0	P_2O_5	as Na_2HPO_4
2.0	Sn	as $SnCl_2$	2.0	SiO_2	as $Na_2Si_4O_9$
5.0	NO_3	as KNO_3	2.0	Fe	as $Fe_2(SO_4)_3$

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† Ohio Agricultural Experiment Station, Wooster, Ohio.

DETERMINATION OF PHOSPHORUS IN FRUITS AND FRUIT PRODUCTS*

By H. W. Gerritz (U. S. Food and Drug Administration, San Francisco, Calif.)

The method presented has been found by the writer to be more convenient and more rapid than the volumetric procedure[1] now frequently used in the estimation of small amounts of phosphate such as are present
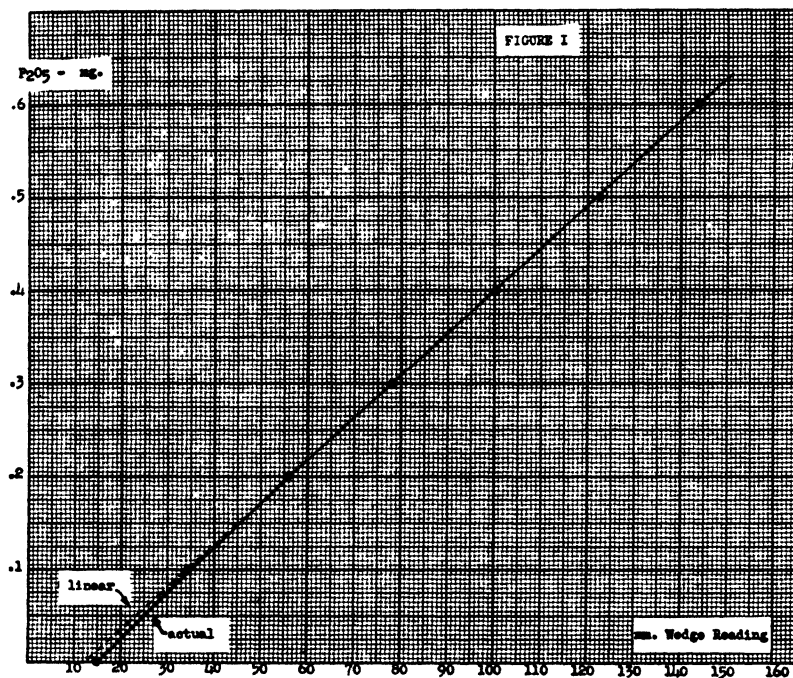
in fruit products. The proposed method also appears to be more accurate than the volumetric method for the determination of P_2O_5 in such substances as raspberries and raspberry jam, and to compare well with the gravimetric procedure.[2]

The proposed method is essentially the molybdenum blue phosphate determination devised by Zinzadze,[3] combined with a rapid wet-ashing procedure previously described by the writer.[4] Minor modifications have been introduced to adapt Zinzadze's method more specifically to the use of the neutral wedge photometer[5] and to the analysis of samples prepared by wet ashing.

METHOD

REAGENTS

(1) *Zinzadze's reagent.*—Follow exactly the directions and precautions given by Zinzadze[2] noting that a ten-fold dilution is used in the actual determination. Preparation of the reagent is also described in detail by Klein and Vorhes (see p. 122).

(2) *Potassium hydroxide solution.*—Phosphate and arsenate free and approximately 3.6 N. J. T. Baker C. P. sticks labeled as containing 0.000% PO_4 have been found satisfactory. Dissolve the KOH in water, using an arsenic-free Pyrex or porcelain vessel, cool immediately, and transfer to a paraffin-lined container. Avoid leaving glass equipment in contact with this reagent for any extended period.

(3) *Normal potassium hydroxide.*—From Reagent 2 prepare about 50 cc. of approximately normal KOH. Preserve in a paraffined container fitted with a 1-holed rubber stopper bearing a Pyrex medicine dropper.

(4) *Concentrated sulfuric acid.*—Reagent quality.

(5) *Normal sulfuric acid.*—From Reagent 4, prepare about 50 cc. of approximately normal H_2SO_4. Preserve in a container fitted with a 1-holed rubber stopper bearing a medicine dropper.

(6) *Concentrated nitric acid.*—Reagent quality.

(7) *Perchloric acid.*—60%, reagent quality.

(8) *Sodium alizarin sulfonate.*—Dissolve 0.20 gram of sodium alizarin monosulfonate in 100 cc. of water and filter. Preserve in an indicator bottle.

(9) *Standard phosphate solution.*—0.05 mg. per cc. Dissolve 0.1917 gram of pure dry KH_2PO_4 in about 200 cc. of distilled water, add 10 cc. of normal H_2SO_4 and 6 drops of 0.1 N $KMnO_4$. Dilute to exactly 2000 cc. According to Zinzadze[2] this solution keeps indefinitely in a well-stoppered Pyrex bottle.

(10) *Glass beads and broken porcelain.*—Boil a supply of small glass beads (2 or 3 mm. in diameter) and a number of small pieces of broken porcelain in aqua regia, wash clean with distilled water, and dry.

PREPARATION OF SAMPLE

Transfer a portion of the sample containing 1–3 mg. of P_2O_5 to a 500 cc. Kjeldahl flask. For the determination of P_2O_5 on the water-soluble portion of fruits and fruit juices use 25 cc. (equivalent to 3.75 grams) of the sample solution prepared according to the official methods,[2] XXVI (b) or (c). For jams and jellies use 50 cc. of the prepared solution. (If the sample is apparently substandard, a larger aliquot may be taken in order to have sufficient P_2O_5 present in the final determination.) Add 15 cc. of the HNO_3, 5 cc. of the H_2SO_4 (Reagent 4), 5 or 6 small glass beads, and a few small pieces of broken porcelain. Place the flask on a digestion rack over a free flame and protect from the flame by an asbestos mat with a hole of such size that the surface of the H_2SO_4 will be above the mat. Boil over a moderate flame to copious fumes of H_2SO_4. If marked charring occurs, as evidenced by a black foam, cool slightly, and cautiously add a little more of the HNO_3. (In the case of jams, three additions of the acid may be necessary.) Again boil to fumes. Add 0.5 cc. of the $HClO_4$ to the hot flask and continue fuming for a few minutes. The digest should now be water-clear or slightly greenish yellow. If necessary, add another 0.5 cc. of $HClO_4$, and continue fuming 3 or 4 minutes. Cool somewhat, cautiously add 50 cc. of distilled water, and boil to fumes to remove traces of the HNO_3. Cool, add 25 cc. of distilled water, transfer to a 100 cc. volumetric flask, mix, cool, make to volume, and again mix thoroughly.

DETERMINATIONS

Transfer a 20 cc. aliquot to a 100 cc. volumetric flask (a Kohlrausch sugar flask has been found convenient) marked at 60 cc. capacity. Always use a 20 cc. aliquot. Add 3 drops of the sodium alizarin sulfonate and neutralize with the strong KOH. Adjust the acidity to just yellow by means of the normal KOH and normal H_2SO_4, adding each dropwise with constant mixing until a single drop of the H_2SO_4 just changes the color of the solution to yellow.

Dilute to the 60 cc. mark and mix. Place the flask in a boiling water bath and bring to that temperature. If the solution becomes pink, add a drop or two of the normal H_2SO_4 to bring it back to yellow. With a pipet add exactly 10 cc. of Zinzadze's reagent (10-fold dilution). Mix and continue to heat in the boiling water bath for exactly 20 minutes. Cool, dilute to volume, and mix.

NOTE: It is important that the standards and unknowns be heated at the same temperature, and this is readily accomplished by immersing the flasks in a boiling water bath to the depth of the liquid within. A simple boiling water bath may be prepared by placing a coarse wire screen in the bottom of a 14 or 16 inch granite pan, filling the pan with water to such a depth that the 60 cc. level in the flasks will be below the level of the water. Place the pan on a tripod and boil vigorously over a Meeker burner. The flasks should be placed only around the perifery of the pan and should be weighted with lead rings or otherwise supported to prevent tipping. The bath should be kept at a turbulent boil throughout the heating period and *boiling* water should be added to the bath from time to time to keep the level of the water equal to or above the level of the liquid in the flasks.

Determine the color intensity by means of the neutral wedge photometer (5), using a 1-inch cell and No. 66 filter,* and Jena 0–2 neutral wedge.

The method covers a range up to 0.6 mg. of P_2O_5 in the final 100 cc. of solution. Prepare standards covering this range by placing 0, 2, 4, 6, 8, 10, and 12 cc. of the standard phosphate in 100 cc. volumetric flasks marked at 60 cc. capacity. Add 2 cc. of H_2SO_4 (1 +1) and 3 drops of the indicator to each flask, together with sufficient water to make about 20 cc. Then treat the same as the sample beginning with the neutralization, dilute to 60 cc., develop color, cool, make to volume, and determine color intensity in the neutral wedge photometer. If alkali of suitable purity has been used the 0 standard should give a reading not over 10–15 mm. greater than the reading of pure water. Make a large scale graph of the standards, plotting mg. of P_2O_5 against photometer readings. By means of this plot convert the sample photometer readings to mg. of P_2O_5 present in the final 100 cc. portion.

NOTES

The photometer need be calibrated but once for each batch of reagents provided the adjustment is not altered.

It will be noted that standardization under these conditions automatically corrects for the blank on reagents, except HNO_3 and $HClO_4$. These acids, in reagent grade, have not been found to contain significant quantities of arsenates or phosphates.

In the above procedure the equivalent of 1 cc. of concentrated H_2SO_4 is present in the final flask in which the color is to be developed. It is important to have approximately the same conditions in both the standard and sample flask. For that reason 2 cc. of H_2SO_4 (1 +1) is added to the standard flasks.

In the analysis of heterogeneous samples, such as those of fresh fruit, for total P_2O_5, it may be necessary to digest a larger portion in order to eliminate sampling

* Filter 66 is 4.5 MM Corning dark pyrometer red No. 241. With B & L "Smoke C" glass wedge, use filter 65. Filter 65 is the same as 66 plus a half MM of Jena BG18.

and weighing error. In such cases it is convenient to take double the sample (7.5 grams) and add double the amount of H_2SO_4 (or 10 cc.). Make the digest to 200 cc., and finally transfer a 20 cc. aliquot to a 100 cc. volumetric flask for color development. The amount of sample digested may be varied to suit the nature of the sample so long as the final 20 cc. aliquot taken for color development contains approximately 1 cc. of H_2SO_4 and 0.2–0.6 mg. of P_2O_5.

EXPERIMENTAL

With his method of sample preparation, Zinzadze[3] found it preferable to develop the color on a steam bath in 30 minutes.

Klein and Vorhes (see p. 121), using Zinzadze's reagent in estimation of arsenic, preferred to develop the color by direct boiling for 5 minutes and obtained a linear calibration curve by this means. The writer was unable to obtain concordant results by Zinzadze's technic on samples prepared by acid digestion. This may have been due in part to variation of temperature in various steam baths and in various locations of a single steam bath. It appears more likely, however, that the relatively higher salt concentration in the proposed procedure is responsible.

A fairly satisfactory calibration curve was obtained by direct boiling for 5 mintues, but here again the higher salt concentration interfered, causing uneven boiling and spattering. To avoid these difficulties, the writer selected the boiling water bath as being more reproducible and less liable to mechanical difficulty. Table 1 presents data on the basis of which the 20-minute period was selected as giving the more nearly linear, and therefore the optimum, calibration curve.

TABLE 1

MILLIGRAMS P_2O_5	BLANK	0.1	0.2	0.3	0.4	0.5	0.6
			Wedge Reading—mm.				
Boiled 5 min. on hot plate	13.9	35.5	56.0	78.0	99.2	120.7	142.3
15 min. in boiling water bath	13.3	33.2	54.5	77.2	99.0	119.0	131.9
20 min. in boiling water bath	15.0	34.6	56.0	78.0	100.1	122.7	144.0
30 min. in boiling water bath	17.2	38.0	55.0	77.0	98.0	120.0	142.1
60 min. in boiling water bath	23.8	40.9	58.6	80.5	99.4	121.0	—

A typical graph, plotting MM wedge reading against milligrams of P_2O_5 in standards is given in Figure 1. It will be noted that the points from 0.1 to 0.6 mg. tend to fall on a straight line, but when this line is projected through the origin, it does not pass through the point experimentally obtained for the blank. Apparently the time-concentration effect is greater in the lower region. For this reason blanks cannot readily

be taken care of numerically and it is imperative that the reagents be as free from P_2O_5 as possible and that the reagents used in calibration of the photometer be the same as those used in the determination. As all reagents used are quite stable, a fairly large supply of each is preferably prepared at the time of calibration of the photometer.

RECOVERY OF P_2O_5 IN MONOBASIC POTASSIUM PHOSPHATE

Reagent quality KH_2PO_4 was dried for 6 weeks in a desiccator over Dehydrite and assayed gravimetrically according to official methods,[2] II, 9; volumetrically according to official methods,[2] II, 12(b), and colorimetrically according to the proposed method. For the colorimetric method 1.5, 2.0, and 2.5 mg. of P_2O_5 were placed in a digestion flask together with a piece of filter paper, 5 cc. of H_2SO_4, and 15 cc. of HNO_3. The digestion and determination were then conducted according to the proposed procedure. Results are given in Table 2. They show that with pure solutions the gravimetric and colorimetric methods give practically 100 per cent recovery, while the volumetric method gives slightly more than 100 per cent recovery of P_2O_5.

TABLE 2

METHOD	P_2O_5 TAKEN	P_2O_5 RECOVERED	
	mg.	*mg.*	*per cent*
Gravimetric	78.77	78.84	100.1
	78.77	78.52	99.7
	78.77	78.97	100.3
Volumetric	7.33	7.42	101.2
	8.80	8.92	101.4
Colorimetric	1.5	1.51	100.6
	2.0	2.01	100.4
	2.5	2.50	100

COLLABORATIVE WORK

Two samples of raspberries and two samples of raspberry jam were supplied to collaborators with the request that they conduct P_2O_5 determinations on the water-soluble portion by the proposed colorimetric method and also by the frequently used volumetric method.[1] When time would allow, samples were also analyzed gravimetrically.[2] Results are given in Table 3.

The proposed colorimetric method has also been found applicable to material of higher P_2O_5 content, such as semolina macaroni. Analyses of three samples of semolina macaroni were made by the volumetric method and the proposed colorimetric method. For the colorimetric determination 0.5 gram of ground, well-mixed sample was taken for analysis. The diges-

tion and estimation of P_2O_5 were conducted in the same manner as described above for fruit products. Digestion was completed in 30 minutes. Results by both methods are shown in Table 3.

TABLE 3.*—*Collaborative results*

	H. M. BOLLINGER	H. W. GERRITZ	G. A. PITMAN	L. A. SALINGER	M. G. YAKOWITZ
			P_2O_5 (MG. PER 100 GRAM SAMPLE)		
(1) Raspberry Jam					
Volumetric	30.0–29.4	30.5–30.1	27.5–27.4	29.5–29.3	
Colorimetric	27.7–27.9	27.9–28.0	27.1–27.1	27.0–27.3	
Gravimetric		27.9–28.0		27.3–27.4	
(2) Raspberry Jam					
Volumetric	27.2–26.8	31.0–31.0			29.8–29.7
Colorimetric	28.2–28.3	27.9–27.6			28.3–28.3
Gravimetric		27.7–27.5			
(3) Raspberry Fruit					
Volumetric	64.5–64.5	65.4–65.0			65.1–64.5
Colorimetric	63.3–62.6	62.7–62.7			63.6–63.2
Gravimetric		62.9–61.8			
(4) Raspberry Fruit					
Volumetric	47.5–46.7	48.8–48.2			
Colorimetric	46.1–46.1	47.7–47.5			
(5) Semolina Macaroni					
Volumetric		326 325			327 329
Colorimetric		327 327			
(6) Semolina Macaroni					
Volumetric		289 292			293 297
Colorimetric		297 297			
(7) Semolina Macaroni					
Volumetric					309 306
Colorimetric		306 306			

* All analysts are at the San Francisco Station, U. S. Food and Drug Administration.

INTERFERENCES

Zinzadze[3] mentions iron, nitrate, and arsenic as interferences in the development of the color by means of his reagent. Nitrates are not present in solutions prepared as described, and neither iron nor arsenic is ordinarily present in fruit or fruit products in sufficient quantity to constitute an interference. Were fruit contaminated with spray residue to the extreme of, say, 0.1 grain As_2O_3 per lb., it would make a positive error in the phosphate determined of 1.4 mg. per 100 grams, which is not a serious error.

However, if the presence of excessive arsenic or iron is suspected, their

interference is prevented by modifying the procedure as follows: Proceed according to the above procedure to the point "adjust acidity to just yellow," then add 10 cc. of exactly normal sulfuric acid and 10 cc. of 8 per cent sodium bisulfite, and dilute to 60 cc. Then heat in a boiling water bath for an hour. Proceed as directed above, beginning "add exactly 10 cc. of Zinzadze's reagent." Standards, of course, must then be treated in the same manner.

CONCLUSIONS

The proposed colorimetric method was found to be more rapid than the volumetric method. The maximum digestion time was about one hour. The proposed method necessitates less manipulation and therefore entails less possibility of error from variation in technic than does the volumetric method. Collaborators' results by the proposed colorimetric method vary less than results by the volumetric method and results by the proposed colorimetric method agree very closely with results obtained by the gravimetric method. On some samples results by the volumetric method appear as a rule to be slightly high.

ACKNOWLEDGMENT

The writer wishes to thank Frank Vorhes, Jr., for helpful suggestions during the course of the work and for enlisting the help of collaborators, and also the collaborators for their cooperation in making the comparative data possible.

DECOMPOSITION OF DOLOMITIC LIMESTONE IN SOILS WHEN USED AS A NEUTRALIZING AGENT IN COMPLETE FERTILIZERS*

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(Soil Fertility Investigations, Bureau of Plant Industry, U. S. Department of Agriculture)

This paper reports preliminary results of studies on the comparative decomposability of dolomitic limestone of different degrees of fineness when applied to soils as the neutralizing agent in non-acid-forming fertilizers. The experiments now in progress comprise pot trials in which the dolomitic materials are applied to the soil as constituents of a complete neutralized fertilizer and are allowed to react with the soil in the presence of a growing crop under conditions simulating those in the field. The comparative rates of decomposition of the dolomites are evaluated by the

changes in pH, the residual carbonates, and the readily soluble magnesium in the soil of the fertilizer placement zone, as well as by the changes in magnesium content of the crop, after each of several successive treatments and crop periods. Taylor and Pierre[1,2] have reported some data of this character; and the purpose of these studies is to extend the information to a wider range of soils and applied treatments.

The preliminary data reported here relate to the effects of a single application of the treatments on the pH and residual carbonates of the soil and on the magnesium content of the crop, following a single crop of corn, and with periods of contact of soil and treatments of 77 to 84 days.

The procedure in these experiments was practically the same as that followed by Taylor and Pierre.[2] The soils used were a Caribou loam of pH 4.6 from Maine, a Sassafras loam of pH 5.0 from New Jersey, and a Sassafras fine sandy loam of pH 5.0 from the Eastern Shore of Virginia. The treatments were based on a 6–8–6 fertilizer, compounded neutral with dolomite. These are listed in Table 1. The hydrated dolomite, applied at 1/3, 2/3, and the full computed rates, constituted the standard of comparison. Composite dolomite A was composed of equal parts of the different dolomite A fractions; dolomites B and C were composed of equal parts of corresponding fractions of dolomites of greater and less reactivity, respectively, than dolomite A. All the magnesic components of the treatments remained segregated from the fertilizer until application to the soil.

The trials were conducted in the greenhouse, in 2-gallon glazed stoneware crocks, holding, on an oven-dry basis, 8500 grams of the Caribou loam and 10,000 grams of the other two soils. The fertilizer and magnesium treatments were applied by thorough mixing with a tenth of the total soil for each pot and inserting this soil as a vertical cylindrical core in the center of the crock. This was accomplished as follows: A hollow metal cylinder, the internal diameter of which was such that one-tenth of the soil in the pot was enclosed and the height of which was somewhat greater than the depth of the soil, was inserted in the center of the empty crock. The nine-tenths weight of untreated soil was placed in the pot outside of the cylinder and compacted by gentle tamping. The treated soil was then packed inside the cylinder and the latter withdrawn. A circular metal collar, 2 inches in height and 3 inches in diameter, protected with asphaltum paint, was centered about the cylinder before complete withdrawal of the latter and embedded about 0.5 inch in the soil. This served to mark the fertilized zone.

The rates of application of the fertilizer and magnesic materials were equivalent to 2000 lbs. of the complete neutralized fertilizer per acre, on the basis of the total soil in the pots; of this total rate of application the magnesium material accounted for an average of 539 lbs. per acre in the

[1] *J. Am. Soc. Agron.*, 27, 764–73 (1935).
[2] Proc. 1st Annual Meeting, Committee on Fertilizers, Am. Soc. Agron., 1935, pp. 15–23.

case of Dolomite A, and equivalent proportions for the other treatments. The initial concentrations in the placement zones (cores) were of course 10-fold these rates. Each treatment was in triplicate for each soil. Corn was planted in the no-fertilizer zone, just outside the core, and allowed to grow for 56–59 days, when it was cut off at the surface of the soil, weighed, and dried for subsequent analysis. Growth was relatively uniform and heavy. The soils were maintained at an average moisture content of 60 per cent of their water-holding capacity by frequent additions of distilled water. After the crop had been harvested the pots were allowed to stand for an additional period of 2–3 weeks, depending on the soil, and during which time the moisture content of the soils was allowed to decrease sufficiently to facilitate sampling. The total intervals between application of the treatments and sampling of the soil were 79 days for the Caribou loam, 77 days for the Sassafras loam, and 84 days for the Sassafras fine sandy loam. During these periods, which extended from the last week of April to the last week of July, the greenhouse temperatures ranged from a minimum of 65°F. at night to a maximum of nearly 100°F. at midday in the latter parts of the periods.

After expiration of these periods of contact of treatments and soil, the fertilizer placement zones (cores) were sampled by means of a metal tube about 1.25 inches in diameter forced down through the entire depth of the centers of the cores. These samples, averaging, on a dry basis, 160–200 grams each, depending on the soil, were air-dried and screened through a 2 mm. sieve.

The pH determinations were made on each of the individual samples for the replicated treatments. Since the results indicated comparative uniformity of the samples for each treatment, 50-gram subsamples of each replicate were composited and ground to 100 mesh for the carbonate analyses. After drying, the entire crop samples for each treatment were combined and ground in a Wiley mill.

The pH values reported were determined on the unleached soils with a glass electrode, 10 grams of air-dry soil to 5 ml. of water being used. The residual carbonates were determined on subsamples of the 100-mesh composites by boiling with hydrochloric acid (1+4) in a Knorr apparatus and absorption of the carbon dioxide in ascarite. The results were corrected for the "blanks" determined on the untreated soils (Treatment 1). The magnesium in the crop samples was evaluated by appropriate conventional methods.

The pertinent data are summarized in Table 1. These results are to be considered as preliminary and of an orienting nature. Interpretation and definite conclusions are deferred until completion of the experiments, when data for the succeeding repetitions of the fertilizer and crop treatments and for the final residual effects are available. A number of comments are, however, appropriate.

TABLE 1.—*Decomposition of dolomitic limestone in the soil of the fertilizer placement zone as evaluated from the pH and residual carbonates, and from the increased magnesium content of the plants, after one crop of corn*

Original pH of soils: Caribou loam, 4.6; Sassafras fine sandy loam, 5.0; Sassafras loam, 5.0

Period of contact with soils: Caribou loam, 79 days; Sassafras fine sandy loam, 84 days; Sassafras loam, 77 days

TREATMENT NO.	FERTILIZER	MAGNESIUM MATERIAL	EFFECT ON pH OR UNLEACHED SOIL, FERTILIZER ZONE						DECOMPOSITION OF DOLOMITIC MATERIALS AS EVALUATED FROM RESIDUAL CARBONATES IN PLACEMENT ZONE				PROPORTION OF MAGNESIUM FROM TREATMENTS RECOVERED IN CROP			RELATIVE RECOVERY OF ADDED Mg
			pH AFTER REACTION PERIOD			RELATIVE EFFECT ON pH, FULL RATE HYDRATED DOLOMITE=100											
			CARIBOU LOAM	SASSAFRAS FINE SANDY LOAM	SASSAFRAS LOAM	CARIBOU LOAM	SASSAFRAS FINE SANDY LOAM	SASSAFRAS LOAM	CARIBOU LOAM	SASSAFRAS FINE SANDY LOAM	SASSAFRAS LOAM	AVERAGE	CARIBOU LOAM	SASSAFRAS FINE SANDY LOAM	SASSAFRAS LOAM	SASSAFRAS FINE SANDY LOAM
									per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1	None	None	4.6	5.1	5.0	—	—	—	—	—	—	—	—	—	—	—
2	0-8-8	None	4.7	4.6	4.8	—	—	—	—	—	—	—	—	—	—	—
3	8-8-8	None	4.3	4.5	4.5	—	—	—	—	—	—	—	—	—	—	—
4	8-8-8	Hydrated dolomite, 1/3 rate	4.5	5.1	4.8	—	—	—	—	—	—	—	4.0	26.1	30.6	—
5	8-8-8	Hydrated dolomite 2/3 rate	4.6	5.6	5.1	—	—	—	—	—	—	—	6.4	22.1	24.3	—
6	8-8-8	Hydrated dolomite, full rate	4.7	6.2	5.4	—	—	—	—	—	—	—	2.1	20.5	7.9	—
7	8-8-8	Dolomite A; 20-40 mesh	4.4	4.8	4.7	16	17	23	15.4	17.2	8.5	13.7	—	1.2	7.9	5.9
8	8-8-8	Dolomite A; 40-60 mesh	4.6	5.2	4.9	71	40	43	31.9	23.3	41.4	32.2	1.4	2.9	1.9	14.1
9	8-8-8	Dolomite A; 60-80 mesh	4.7	5.4	5.0	100+	53	53	61.2	23.4	41.4	42.0	4.7	6.3	10.8	30.7
10	8-8-8	Dolomite A; 80-100 mesh	4.7	5.5	5.1	100+	58	64	67.3	36.4	55.3	53.0	1.5	8.9	17.2	43.4
11	8-8-8	Dolomite A; 100-200 mesh	4.9	5.5	5.3	100+	58	85	87.0	48.2	68.1	67.8	4.0	0.4	9.7	45.9
12	8-8-8	Dolomite A; 200 mesh	4.9	5.9	5.5	100+	83	108	96.5	58.5	87.9	81.0	7.7	12.3	13.4	60.0
13	8-8-8	Selectively calcined dolomite	4.8	6.3	5.4	—	—	—	—	—	—	—	8.0	20.3	18.9	—
14	8-8-8	Magnesium sulfate equiv. to Mg in No. 4	4.3	4.6	4.5	—	—	—	—	—	—	—	2.6	10.8	19.6	—
15	8-8-8	Magnesium sulfate equiv. to Mg in No. 5	4.3	4.7	4.5	—	—	—	—	—	—	—	0.2	11.5	11.0	—
16	8-8-8	Magnesium sulfate equiv. to Mg in No. 6	4.3	4.7	—	—	—	—	—	—	—	—	4.4	15.6	—	—
17	8-8-8	Composite dolomite A	4.7	5.4	5.1	100+	83	64	61.2	42.2	44.8	49.4	3.4	9.4	7.5	45.9
18	8-8-8	Composite dolomite B	4.7	5.5	5.2	100+	58	75	67.3	43.4	51.4	54.0	0.4	12.3	5.6	60.0
19	8-8-8	Composite dolomite C	4.6	5.4	5.0	71	83	83	47.1	32.4	41.1	40.2	3.4	5.1	12.9	24.9

* Rates of application equivalent to 2000 lbs. of neutralized fertilizer per acre: confined to one tenth of total weight of soil.
† Assigning value of 100 to proportion of added magnesium recovered in crop with full rate of hydrated dolomite.

The changes in pH resulting from the several treatments are relatively consistent; but the range of variation differs in magnitude for each of the three soils. It is narrowest for the highly buffered Caribou loam, widest for the poorly buffered Sassafras fine sandy loam, and intermediate for the intermediately buffered Sassafras loam. The "relative effect on pH" was derived by the procedure of Taylor and Pierre, by comparing the values for the several dolomites with those for the hydrated dolomite, and assigning values of 33-1/3, 66-2/3 and 100 per cent to the 1/3, 2/3 and full rates of the latter, respectively. While these derived figures are relatively consistent in trend, the indicated complete decomposability of the 60–80 mesh and finer material, in contact with the Caribou loam, should be accepted with reservation, even though higher reactivity in this more acid soil would be anticipated. The range of pH variation in the case of the well-buffered Caribou loam is so narrow as to lessen the significance of the absolute numerical magnitudes of derived values.

The values for decomposition of the dolomitic materials, as derived from the values for residual carbonates, are generally consistent with the treatments and with the characteristics of the soils; and they are in harmony with the values derived from the pH data. For a given dolomite the rate of decomposition is highest in the Caribou loam, lowest in the Sassafras fine sandy loam, and intermediate in the Sassafras loam.

Data are also presented for the percentages of applied magnesium recovered in the crops. These are computed from the increases in magnesium content of the crops grown with magnesium supplements as compared with the content of the crop with the complete acid fertilizer (Treatment 3), assuming such increases to be attributable to the magnesium added in the treatments. Such comparisons are the only appropriate ones available within the range of treatments used, but they involve the question of effect of variations in the pH and calcium content of the soil on absorption of magnesium by the crop. Only in the case of the Sassafras fine sandy loam are the results consistent. For this soil an approximate index of the decomposition of the several dolomites is found in the "relative recovery of added magnesium," derived by expressing the values for the percentages of added magnesium recovered in the crop as proportions of the corresponding value for the full rate of hydrated dolomite, assigning the latter a value of 100. These comparisons are made with reservations, but the resulting values are rather consistent with those derived from the residual carbonates.

Further studies will include evaluation of the increase in readily soluble magnesium of the soil associated with the several dolomitic treatments.

These preliminary data indicate that, for the present, the residual carbonates constitute the most satisfactory index of the comparative rates of decomposition of the different dolomitic materials.

DECOMPOSITION OF DOLOMITIC LIMESTONE IN SOILS WHEN USED AS A NEUTRALIZING AGENT IN COMPLETE FERTILIZERS*

STUDIES ON DUNBAR VERY FINE SANDY LOAM, RUSTON SANDY LOAM, NORFOLK FINE SANDY LOAM, AND PORTSMOUTH FINE SANDY LOAM

By E. R. COLLINS and F. R. SPEER[1] (Agronomy Department, North Carolina Experiment Station, Raleigh, N. C.)

This work constitutes part of a program of study to determine the availability of magnesium in dolomitic limestone of different degrees of fineness when used as the neutralizing agent in non-acid-forming fertilizers. The purpose of this study was to determine the rate of decomposition of the dolomitic limestone as evaluated by reaction change in the soil and by the determination of residual carbonates after a crop of cotton had been grown on it for 65–75 days. Magnesium determinations were made on a few plant samples to indicate any change in the concentration of magnesium in the plant due to the decomposition of the dolomitic limestone during the growing season.

OUTLINE OF THE EXPERIMENTS

The general plan of these experiments and method of procedure was the same as that used by Taylor and Pierre.[2] The soils selected were a Dunbar very fine sandy loam of pH 5.45, Ruston sandy loam of pH 5.60, Norfolk fine sandy loam of pH 5.42, and Portsmouth fine sandy loams of pH 5.18 and 4.50. The soils selected were considered to have pH values typical of these soils. The two Portsmouth fine sandy loam soils of different reactions were taken to determine the relationship between the pH value of the soil and the percentage decomposition of the dolomitic limestone. Nineteen treatments were used in duplicate on the five soils comprising four soil types.

METHOD OF PROCEDURE

Each soil type studied was potted in 2-gallon pots in the usual manner, and the soil was weighed so that each pot contained the same amount of air-dried soil. One-tenth of the soil in each pot was removed, and fertilizer calculated to be equivalent to 1333 pounds per acre (on the basis of all the soil in the pot) was thoroughly mixed with this soil. A hollow metal cylinder, the inside diameter of which was 2.5 inches, and which enclosed exactly one-tenth of the area of the inside of the pot, but was slightly longer than the height of the pot, was placed in the center of the empty pot, and the fertilized soil was placed inside this metal cylinder and gently

packed by jolting the pot up and down. The untreated soil was then placed in the pot on the outside of the metal cylinder and packed as was the other portion. A circular metal collar protected with asphaltum paint, 2 inches in height and about 3 inches in diameter, was placed over the end of the cylinder and was embedded about 0.5 inch in the soil. The metal cylinder was then removed, and the circular collar marked the fertilized zone.

TABLE 1.—*Percentage decomposition of dolomitic limestone when used as a neutralizing agent in an acid-forming fertilizer*

		DUNBAR VERY FINE SANDY LOAM	RUSTON SANDY LOAM	NORFOLK FINE SANDY LOAM	PORTSMOUTH NO. 1 FINE SANDY LOAM	PORTSMOUTH NO. 2 FINE SANDY LOAM	AVERAGE
Original pH of the respective samples		5.45	5.60	5.42	5.18	4.50	
Time of contact with soil (days)		75	75	65	72	78	
PLAT NO.	TREATMENT			PER CENT DECOMPOSITION			
7	6-8-6+Dolomite A, 20-40-mesh	22.4	17.8	30.4	29.3	37.4	27.5
8	6-8-6+Dolomite A, 40-60-mesh	52.2	33.1	30.4	51.5	63.3	46.1
9	6-8-6+Dolomite A, 60-80-mesh	64.0	42.7	35.4	70.7	87.4	60.0
10	6-8-6+Dolomite A, 80-100-mesh	67.7	45.9	41.8	79.9	94.0	65.9
11	6-8-6+Dolomite A, 100-200-mesh	83.2	58.0	55.7	94.5	99.4	78.2
12	6-8-6+Dolomite A, through 200-mesh	87.6	75.2	76.0	97.6	100.0	87.3
16	6-8-6+Composite A[1]	59.0	45.9	36.7	70.7	80.1	58.5
17	6-8-6+Composite B[1]	70.1	53.3	44.3	78.1	83.9	65.9
18	6-8-6+Composite C[1]	55.9	38.9	37.3	62.8	79.5	54.9
	Per cent $CaCO_3$ in check soil	0	0.006	0.008	0.0015	0.0007	

All the pots were planted to cotton and kept at optimum moisture content with tap water for 65–75 days. The plants were then removed and dried for further analysis. Soil samples were removed from the fertilizer zone by means of a circular cylinder about 1.25 inches in diameter, which was forced down through the center of the core of fertilized soil.

A portion of each sample was leached for the determination of soil reaction, and the remainder was air-dried for the residual carbonate determination by the Schollenberger method.[1]

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[1] *Soil Sci.*, **30**, 307–325 (1930).

RESULTS

The results[1] in Table 1 show the percentage decomposition of the dolomitic limestone on the five soils of the acidity indicated and for the period of time given. These percentage values were corrected for the check, which received no treatment. Figure 1 shows clearly the difference in decomposition of the various particle sizes. Even the coarser grades were appreciably decomposed during the growing season, the decomposition of the 40–60 mesh dolomite averaging nearly 50 per cent on the five soils and that of the 20–40 mesh 27.5 per cent.
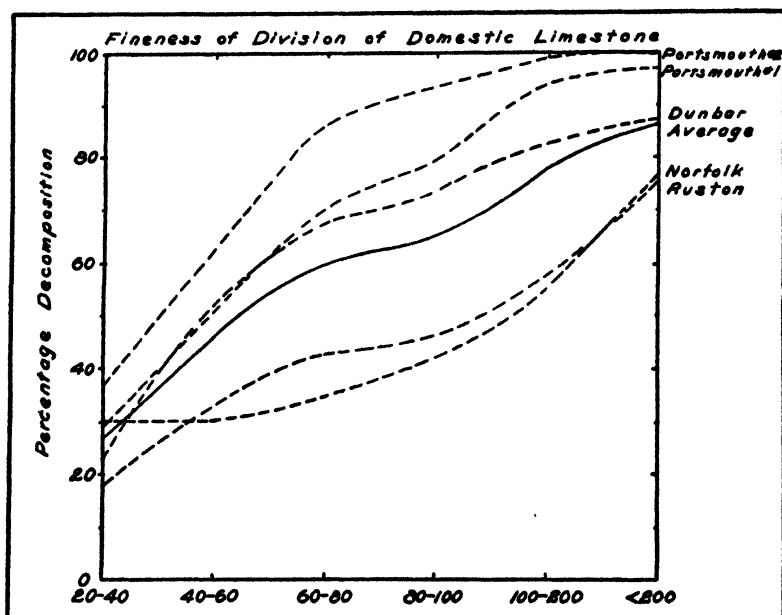
FIG. 1.—PERCENTAGE DECOMPOSITION OF DIFFERENT DOLOMITE SEPARATES
ON FIVE SOIL TYPES

The Portsmouth soils of higher organic matter content, greater buffer capacity, and lower *p*H values show a marked increase in decomposition of all the dolomitic fractions over that of the more sandy and less acid Norfolk and Ruston soils. The Dunbar soil occupies an intermediate position with respect to the other two in the relative decomposition of the various fractions. Composite dolomite B is the most highly reactive of the composite dolomites, while composite A is superior to composite C. This difference in decomposition of the composite dolomites was evident throughout the five soils.

TABLE 2.—*The pH of leached soil after contact with treatments shown for the period indicated*

PLAT NO.	TREATMENT FERTILIZER	MG MATERIAL	DUNBAR VERY FINE SANDY LOAM		RUSTON SANDY LOAM		NORFOLK FINE SANDY LOAM		PORTSMOUTH NO. 1 FINE SANDY LOAM		PORTSMOUTH NO. 2 FINE SANDY LOAM	
			1	2	1	2	1	2	1	2	1	2
1	None	None	5.45	5.33	5.77	5.67	5.37	5.37	4.86	4.92	4.57	4.57
2	0-8-6	None	5.29	5.23	5.43	5.43	5.24	5.20	4.93	4.87	4.43	4.43
3	6-8-6	None	4.84	4.77	5.29	5.26	4.99	5.03	4.84	4.86	4.29	4.29
4	6-8-6	1/3 rate hydrated dolomite	4.92	4.99	5.29	5.45	5.13	5.03	4.99	5.07	4.35	4.27
5	6-8-6	2/3 rate hydrated dolomite	5.14	5.07	5.60	5.63	5.17	5.29	5.03	5.04	4.33	4.29
6	6-8-6	Full rate hydrated dolomite	5.21	5.28	5.80	5.87	5.65	5.72	5.27	5.20	4.46	4.46
7	6-8-6	Dolomite A, 20–40 mesh	4.87	4.92	5.51	5.51	5.13	5.13	4.88	4.82	4.57	4.61
8	6-8-6	Dolomite A, 40–60 mesh	5.02	4.97	5.63	5.63	5.15	5.14	5.01	4.98	4.58	4.60
9	6-8-6	Dolomite A, 60–80 mesh	5.06	5.12	5.82	5.82	5.25	5.23	5.04	5.06	4.62	4.67
10	6-8-6	Dolomite A, 80–100 mesh	5.16	5.16	5.80	5.80	5.22	5.33	5.14	5.23	4.65	4.64
11	6-8-6	Dolomite A, 100–120 mesh	5.26	5.23	5.99	6.07	5.33	5.31	5.25	5.26	4.62	4.68
12	6-8-6	Dolomite through 200 mesh	5.29	5.41	6.03	6.17	5.53	5.57	5.29	5.22	4.75	4.68
13	6-8-6	Selectively calcined dolomite	5.50	5.51	6.51	6.38	5.73	6.00	5.24	5.18	4.57	4.57
14	6-8-6	MgSO$_4$⇌to Mg in No. 4	5.02	5.02	5.63	5.33	5.47	5.28	4.69	4.65	4.29	4.29
15	6-8-6	MgSO$_4$⇌to Mg in No. 5	4.84	4.84	5.33	5.43	5.11	5.20	4.55	4.56	4.33	4.25
16	6-8-6	Composite dolomite A*	5.12	5.12	5.43	5.39	5.38	5.32	4.91	4.95	4.43	4.43
17	6-8-6	Composite dolomite B	5.23	5.23	5.56	5.70	5.38	5.51	5.01	5.00	4.45	4.45
18	6-8-6	Composite dolomite C	5.02	5.06	5.53	5.53	5.21	5.25	5.17	4.87	4.40	4.30
19	6-8-6	MgSO$_4$⇌to Mg in No. 6	4.79	4.79	5.14	5.14	5.15	5.13	4.63	4.58	4.23	4.25
	Original pH of soils		5.45		5.60		5.42		5.18		4.50	
	Time of contact with soil (days)		75		75		65		72		78	

* Composite dolomite A, B, and C represent material from different quarries, and each one was composed of equal parts of 20–40, 40–60, 60–80, 80–100, 100–200, and through 200 mesh dolomite.

TABLE 3.—*Magnesium content of cotton crop*

TREATMENT NO.	FERTILIZER	Mg MATERIAL	DUNBAR MgO	DUNBAR Ca-Mg RATIO	NORFOLK MgO	NORFOLK Ca-Mg RATIO	PORTSMOUTH NO. 1 MgO	PORTSMOUTH NO. 1 Ca-Mg RATIO	RUSTON MgO	RUSTON Ca-Mg RATIO
			per cent		*per cent*		*per cent*		*per cent*	
1	None	None	1.39	1.89	0.78	3.45	0.89	2.15	0.52	3.12
2	0-8-6	None	0.94	2.18	0.62	3.69	0.93	2.51	0.48	3.52
3	6-8-6	None	0.97	2.49	0.63	3.81	1.07	2.09	0.44	4.14
4	6-8-6	1/3 rate hydrated dolomite	1.14	2.12	0.76	3.05	1.09	2.08	0.55	2.71
5	6-8-6	2/3 rate hydrated dolomite	1.18	1.96	0.80	2.80	1.16	1.90	0.67	2.27
6	6-8-6	Full rate hydrated dolomite	1.18	1.94	0.85	2.71	1.14	2.20	0.66	2.08
7	6-8-6	20-40 mesh dolomite	1.16	2.12	0.67	3.40	1.08	2.40	0.42	3.86
8	6-8-6	40-60 mesh dolomite	1.12	2.33	0.70	3.20	1.00	2.16	0.46	3.15
9	6-8-6	60-80 mesh dolomite	1.06	2.38	0.80	3.26	1.07	2.21	0.42	4.10
10	6-8-6	80-100 mesh dolomite	1.20	2.00	0.79	2.99	1.22	1.99	0.57	2.63
11	6-8-6	100-200 mesh dolomite	1.25	1.83	0.80	2.40	1.30	2.22	0.53	2.85
12	6-8-6	Through 200 mesh dolomite	1.30	1.85	0.76	2.58	1.28	1.87	0.69	2.25
13	6-8-6	Selectively calcined dolomite	1.30	1.81	0.91	2.43	1.34	2.06	0.64	2.30
14	6-8-6	$MgSO_4$ ⇌ to Mg in No. 4	1.08	2.17	0.75	2.65	1.06	2.55	0.56	2.73
15	6-8-6	$MgSO_4$ ⇌ to Mg in No. 5	1.20	1.83	0.83	2.77	1.08	2.16	0.51	3.71
16	6-8-6	Composite dolomite A	1.30	2.01	0.74	2.91	1.19	2.03	0.67	2.69
17	6-8-6	Composite dolomite B	1.33	1.79	0.78	3.45	1.35	2.03	0.72	2.25
18	6-8-6	Composite dolomite C	1.25	2.22	0.64	3.56	1.14	2.09	0.66	2.83
19	6-8-6	$MgSO_4$ ⇌ to Mg in No. 6	1.56	1.79	0.94	2.94	1.18	1.93	0.86	1.81

Table 2 gives the *p*H of the leached soil after contact with the fertilizer treatments shown for the period of time indicated. There is considerable variation in the neutralizing value of the several screen fractions on the different soil types. On the Dunbar and Norfolk soils the coarser grades were not very effective in neutralizing acidity while on the Portsmouth and Ruston soils even the 40–60 mesh was sufficient to maintain the *p*H. In general the composite dolomites were very effective in maintaining the *p*H of the soil, especially composite dolomite B. After the period of treatment the *p*H of the soil in the pot receiving composite dolomite B as a supplement was, in no case, more than 0.2 *p*H lower than that of the original soil. Selectively calcined dolomite was a very effective neutralizing agent on all soil types, while magnesium sulfate appeared to increase the acidity slightly.

The magnesium determinations on the cotton plants are not finished and therefore no definite conclusions are given at this time. The preliminary results are included in Table 3.

EFFECT OF PARTICLE SIZE ON THE SOLUBILITY OF MAGNESIUM IN DOLOMITE AND MAGNESIC LIMESTONE IN 4 PER CENT CITRIC ACID SOLUTION ADJUSTED TO *p*H 4.0 WITH AMMONIUM HYDROXIDE*

By J. W. KUZMESKI (Agricultural Experiment Station, Amherst, Mass.)

In their report on Magnesium and Manganese in Fertilizers last year[1] J. B. Smith and E. J. Deszyck of the Rhode Island Agricultural Experiment Station proposed the use of a 4 per cent citric acid solution adjusted to *p*H 4.0 with ammonium hydroxide as a solvent for the evaluation of available magnesium in dolomite and magnesic limestone. As stated by Smith, there is no theoretical background for the use of this solvent at the particular *p*H used. Its adoption for this purpose will depend on the agreement between the results obtained with it and those obtained from the determination of plant response in vegetation work. However, the results presented by Smith on the recovery of magnesium from dolomite by the 4 per cent citric acid solution indicate that this solution has more promise as a medium in the determination of available magnesium in materials carrying magnesium in other than water-soluble forms than have the other solvents he used.

The tentative A.O.A.C. method used for determining the neutralizing value of liming materials[1] makes no distinction between coarsely ground and finely ground products. The value obtained by this method is based solely on the total acid-soluble content of magnesium and calcium oxides,

TABLE 1.—*Deviation in percentage of silica, MgO, and CaO present in each of the fractions used*

SAMPLE	AS SAMPLED	MESH				
		60–80	80–100	100–150	150–200	THROUGH 200
A-2 (%)						
Silica	2.00	2.40	2.25	2.25	2.15	1.50
MgO	21.20	21.23	21.37	21.14	21.15	21.28
CaO	31.40	30.70	30.77	30.98	31.16	31.93
Neut. value equiv. to % CaO	59.02	58.67	58.88	58.88	59.02	59.94
A-3 (%)						
Silica	1.25	1.75	1.75	1.50	1.35	1.10
MgO	21.28	21.37	21.28	21.55	21.55	21.28
CaO	31.12	30.84	30.84	30.84	31.12	31.19
A-29 (%)						
Silica	15.50	25.50	24.50	21.00	15.00	12.25
MgO	16.50	13.35	13.81	15.98	17.16	17.02
CaO	28.74	26.67	27.20	27.76	28.53	29.37
Neut. value equiv. to % CaO	50.47	44.16	44.51	49.42	51.17	52.22
A-53 (%)						
Silica	13.50	*	*	18.50	17.00	11.25
MgO	18.29	*	*	15.93	18.74	18.29
CaO	28.53	*	*	28.11	28.74	29.65
Neut. value equiv. to % CaO	50.82	*	*	43.60	47.11	51.17

* Sample practically all finer than 100 mesh. Not enough material coarser than 100 mesh available to make tests.

hydroxides, and carbonates. Therefore, it was thought that it might be of interest to ascertain the relative solubility of several brands of magnesic limestone and dolomite by using the method suggested by Smith. (It must be stressed that "solubility" as the term is used here is not synonymous with "availability" since, at this time, there is no proof that the solubility of magnesium in this particular solution represents the portion of the magnesium readily available to plants.)

Accordingly, nine samples, each representing a different brand of magnesic limestone or dolomite sold in Massachusetts in 1937, were taken for

analysis. Each sample was sieved, and fractions representing meshes 60–80, 80–100, 100–150, 150–200, and finer than 200, respectively, were used in an effort to determine, if possible, at what particle size the magnesium in the samples would be completely dissolved in the solvent specified. The finest fraction used by Smith was 100–120 mesh.

The method employed by Smith was followed here; 0.2 gram samples were digested in 100 cc. of 4 per cent citric acid, and adjusted to *p*H 4.0 with ammonium hydroxide, at 90–95° C. for 90 minutes, the samples being shaken at intervals of 5 minutes. After filtering and washing, the magnesium was determined in the insoluble residue.

TABLE 2.—*Comparison of total acid-soluble MgO with MgO not dissolved in adjusted 4% citric acid solution*

SAMPLE	ACID-SOLUBLE MgO	PERCENTAGE OF MgO NOT DISSOLVED IN CITRIC ACID-AMMONIUM CITRATE SOLUTION ADJUSTED TO pH 4.0					
		AS SAMPLED	60–80	80–100	100–150	150–200	THROUGH 200
	per cent						
A-2	21.20	6.61	9.64	8.15	4.98	1.77	0.18
A-3	21.28	9.42	9.51	6.88	4.71	1.81	0.18
A-6	19.34	4.53	7.20	5.02	3.30	0.32	0.05
A-12	21.05	6.79	11.91	9.87	7.20	4.48	0.36
A-22	21.70	5.66	8.51	7.15	4.30	1.58	0.18
A-29	16.50	4.53	2.17	1.95	2.53	2.26	0.18
A-53	18.29	3.49	—	—	2.90	2.63	0.81
A-71	12.60	3.40	6.11	4.57	3.58	1.45	0.36
A-74	21.33	5.47	10.86	9.10	6.47	1.49	0.18
Average	19.25	5.54	8.24	6.59	4.44	1.98	0.28

The results obtained, as was expected, ran more or less uniformly in the same direction. That is, the solubility increased with the increase in fineness. There was one exception. Sample A-29 showed a higher percentage of undissolved magnesium oxide in the solvent used in both the 100–150 and the 150–200 mesh fractions than in the 60–80 and 80–100 mesh fractions. It was noted that this sample contained a relatively high percentage of silica (15.50). The discordant results for this sample indicated that the same proportion of silica, magnesium, and calcium was not being maintained in each fraction of the sample. To test this point, the total acid-soluble magnesium oxide and calcium oxide and the insoluble silica were determined in each fraction of this sample, as well as of three other samples. One of the latter was also high in silica, and the other two contained 1–2 per cent. The neutralizing value was determined for each fraction of three of these samples by the A.O.A.C. method.

It was found (Table 1) that in the samples containing a high percentage of silica the percentage content of acid-soluble magnesium oxide and cal-

cium oxide and of total silica varied considerably with each fraction. In the finer fractions of the sample, the percentage of silica decreased while the percentage of calcium oxide and magnesium oxide increased, but the increase was somewhat greater for magnesium oxide. No significant variation in this respect was found in the fractions of the samples low in silica.

Table 2 shows the percentage of magnesium oxide not dissolved by the adjusted 4 per cent citric acid solution, in comparison with the percentage of total acid-soluble magnesium oxide.

TABLE 3.—*Comparison of total acid-soluble MgO with MgO dissolved in adjusted 4% citric acid solution*

SAMPLE	ACID- SOLUBLE MgO	PERCENTAGE OF ACID-SOLUBLE MgO DISSOLVED IN CITRIC ACID-AMMONIUM CITRATE SOLUTION, pH 4.0					
		AS SAMPLED	60-80	80-100	100-150	150-200	THROUGH 200
	per cent						
A-2	21.20	69	55	62	77	92	99.2
A-3	21.28	56	55	68	78	92	99.2
A-6	19.34	77	63	74	83	98.4	99.7
A-12	21.05	68	43	53	66	79	98.3
A-22	21.70	74	61	67	80	93	99.2
A-29	16.50	73	87	88	85	86	99.0
A-53	18.29	81	—	—	84	86	95.6
A-71	12.60	73	52	64	72	88	97.1
A-74	21.33	74	49	57	70	93	99.2
Average	19.25	71	57	66	77	90	98.6

Table 3 gives the percentage of acid-soluble magnesium oxide that was dissolved in the adjusted 4 per cent citric acid solution. The solubility of magnesium varies not only with each fraction of the *same* sample, but also with the same fraction of *each* sample. For example, in the fraction 60–80 mesh the lowest solubility is 43 per cent in Sample A-12 and the highest is 87 per cent in Sample A-29; and in the fraction 100–150 mesh, the lowest is 66 per cent while the highest is 85 per cent. As the fineness approaches 200 mesh, the difference in the solubility of magnesium in the several samples becomes smaller, and the magnesium in the fractions finer than 200 mesh is practically all soluble, the average for the nine samples being 98.6 per cent.

The results of this study seem to justify the belief that the solubilities recorded might have a definite ratio to the rate of solubility or availability of the magnesium and calcium in a limestone product following its application to the soil, and they also emphasize the great advantage of fine grinding from the standpoint of immediate effectiveness of the product, both in neutralizing value and in furnishing available plant food.

APPLICATION OF THE NEUTRAL WEDGE PHOTOMETER TO THE QUANTITATIVE DETERMINATION OF METHANOL IN DISTILLED SPIRITS

By G. F. BEYER (Alcohol Tax Unit, Bureau of
Internal Revenue, Washington, D. C.)

Some distilled spirits, such as fruit distillates, normally contain small quantities of methanol, and since it is often necessary to make this determination it seemed to be desirable to develop a method. The only procedure available for such small quantities as 0.01 per cent requires complex as well as cumbersome apparatus and also excessive manipulation.

Since a colorimetric method was considered to be best adapted for this purpose, the Georgia-Morales[1] modification of Deniges'[2] method was selected, except as to the preparation of the sample, because it is the simplest procedure and produces a color that is stable a sufficient length of time to permit readings to be taken in a photometer.

It has long been known that ethyl alcohol definitely affects the sensitiveness of the Georgia-Morales test, but a search of the literature failed to disclose what concentration produces a maximum amount of color. Deniges adds ethyl alcohol to the portion in the test tube; Simmonds[3] uses a 5 ml. sample containing 10 per cent of ethyl alcohol, while Chapin[4] and Georgia and Morales[5] recommend the use of a 5 ml. sample that has previously been diluted to 5 per cent by volume of total alcohols. However, no mention is made of the fact that varying concentrations of ethyl alcohol greatly influence the sensitivity of the test, and therefore it was first necessary to find this optimum percentage of ethyl alcohol.

EXPERIMENTAL

Solutions were made wherein the amount of methanol was kept constant and that of the ethanol was varied. The solutions tested contained 0.08 per cent methanol by volume, while the ethyl alcohol varied from 17.0 to 40.0 per cent by volume and from each other by approximately 2.5 per cent. The color produced by the reactions involved increased in intensity as the percentage of ethyl alcohol decreased, until it was as low as 20.0 per cent. Very little, if any, difference could be noted in the 20.0, 23.0, and 25.0 per cent solutions. The photometer, however, showed that the depth of color obtained from the 20.0 and 23.0 per cent solutions were very slightly deeper than that obtained from the 25.0 per cent solution. However, this difference, may be but slightly outside the limit of experimental error.

The photometer used is known as a Neutral Wedge Photometer and was built around the specifications published by Clifford and Wichmann.[6]

[1] *J. Ind. Eng. Chem.*, **18**, 1312–3 (1926).
[2] *Compt. rend.*, **150**, 832 (1910).
[3] *Analyst*, **37**, 16 (1912).
[4] *J. Ind. Eng. Chem.*, **13**, 543 (1921).
[5] *Ibid.*, **18**, 1312 (1926).
[6] *This Journal*, **19**, 150 (1936).

SELECTION OF PROPER COLOR FILTER

By the use of this photometer and a series of color filters covering the visible spectrum, an abridged spectrograph of the color obtained was made. It is shown in Figure 1. The greatest absorption occurs between 560 and 606 mμ. Hence, if the light filter used transmits only in that spec-
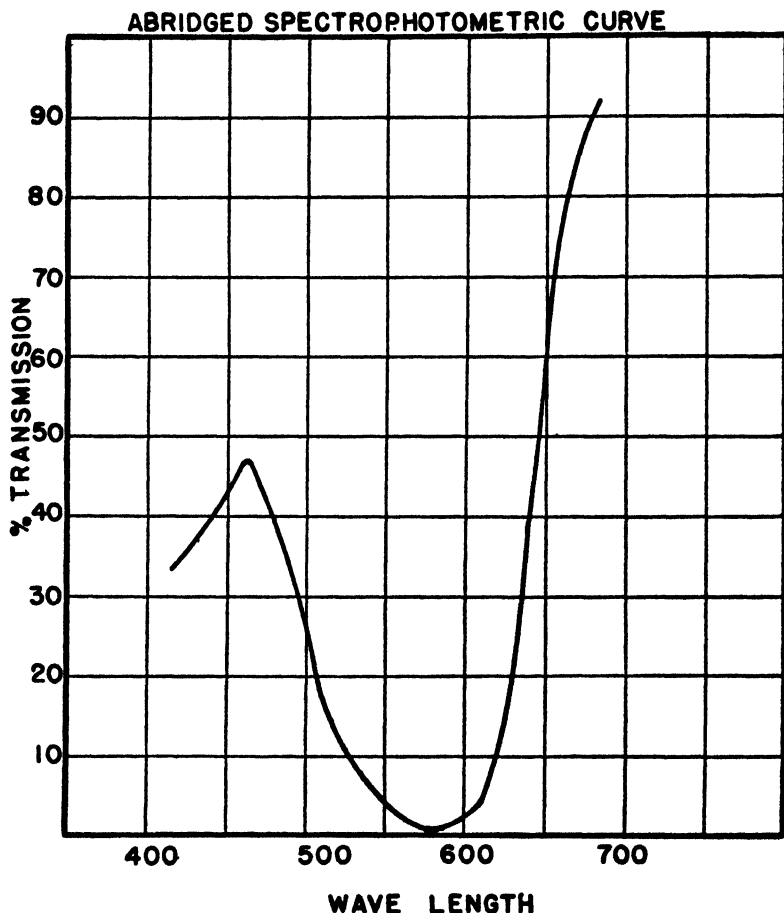
FIG. 1.—ABRIDGED SPECTROPHOTOMETRIC CURVE

tral region, accurate results should be possible, provided the color is sufficiently stable to make the photometer readings. The filters used in this work were those centering around 560, 580, 590, and 610 mμ.

After it had been determined which filters to use, it was then necessary to determine how long the colored solutions should stand to reach a maximum of intensity, how long the color remained stable, and the perma-

nency of the reacting solutions. Various tests showed that the colored solutions must stand about 2 hours before the maximum color is developed and that it remains practically constant for nearly an hour, after which it gradually fades. Numerous tests indicated that the potassium permanganate solution had a tendency to give low results after five weeks, and that a modified Schiff's reagent gave unsatisfactory results after standing four weeks. This solution keeps much better in the cold and when the bottle is fairly well filled. Therefore these solutions should be checked occasionally by testing a sample containing a known quantity of methanol.
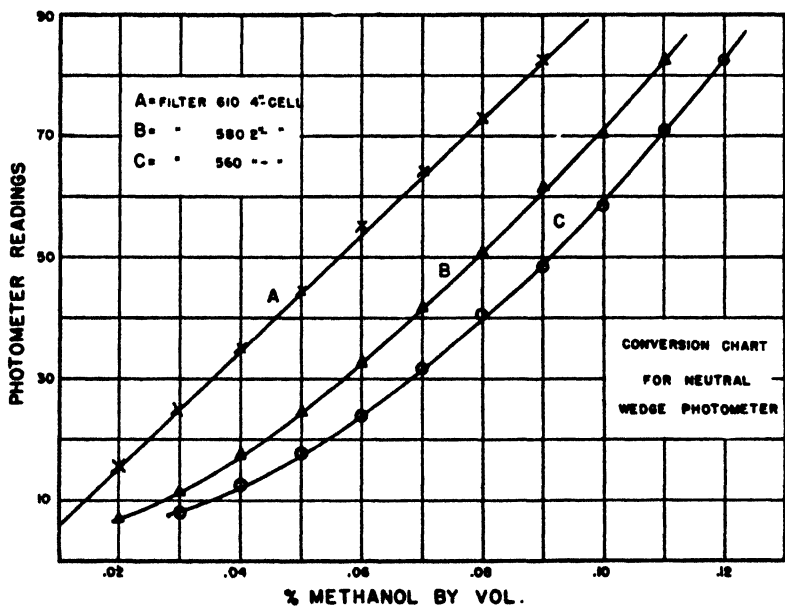
FIG. 2

All of the work with the photometer recorded here was performed in a constant temperature room, which was kept at 25° C. Not more than five readings were taken on any one solution because the lamp used in connection with the photometer throws off considerable heat, which would cause an appreciable loss of sulfur dioxide and thus affect the intensity of the violet color.

Having determined the various optimum conditions for the experiment, the writer made photometer readings on the color obtained from methanol solutions varying in concentrations from 0.02 to 0.15 per cent and from each other by 0.01 per cent. Standard curves were constructed from these readings. Figure 2 shows curves obtained by using filters centering around 560, 580, and 610 mμ.

EFFICIENCY OF STILL USED

A diagram of the upper part of the column and "take-off" of the still is shown in Figure 3.

The packed part of the column of the still, 19 inches long, is filled with small, single-turn, glass helices and is enclosed in a glass tube about 30

CONDENSER ABOUT 8" LONG

15 MM.

CAPACITY OF U-TUBE TAKE-OFF ABOUT 1.2 ML.

CAPILLARY

30 MM.
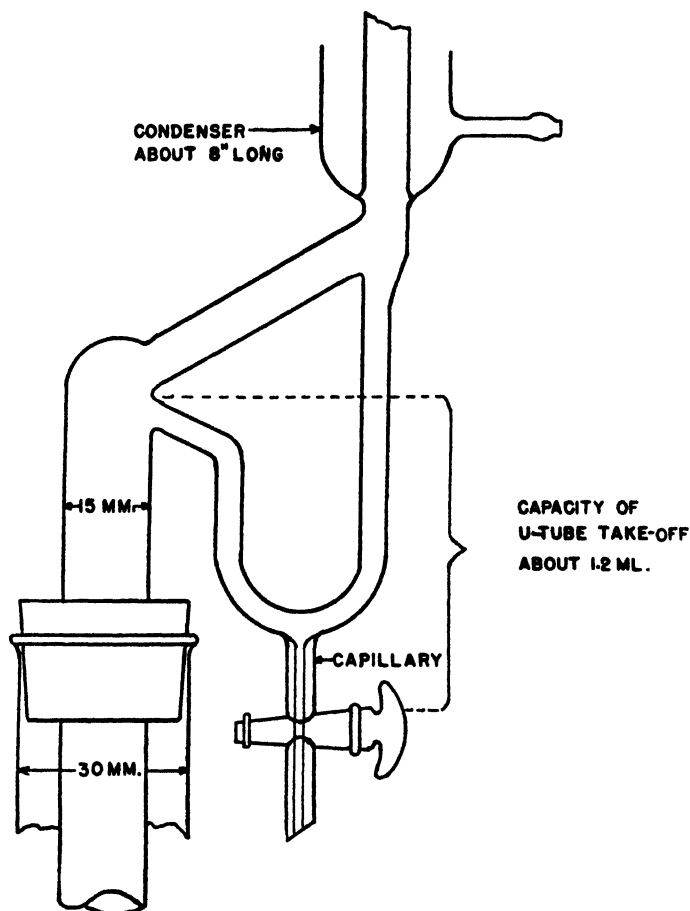
FIG. 3.—DIAGRAM OF UPPER PART OF STILL AND TAKE-OFF

mm. in diameter; 25 ml. of a solution containing 0.1 per cent methanol in 50 per cent ethyl alcohol is placed in a 100 ml. round-bottomed flask connected with the above column and distilled, an electric precision heater being used. After distillation starts the still is allowed to remain under total reflux for about 30 minutes, then fractions consisting of about 1.2 ml. are drawn off at 15 minute intervals until about 8.6 ml. are received. The distillate is transferred to a 50 ml. graduated cylinder, diluted to about 22 per cent total alcohol by volume, then further diluted with

22 per cent ethyl alcohol to 50 ml. Another sample is distilled in the same manner, except the distillate is received in a 50 ml. volumetric flask, but it is similarly diluted. Photometer readings are as follows, and when applied to the curves obtained show the method to be more than 95 per cent accurate.

	2″ Cell		*4″ Cell*	
Filter	Sample 1	Sample 2	Sample 1	Sample 2
	per cent	*per cent*	*per cent*	*per cent*
560	$-18.5 = 0.104$	$18.5 = 0.104$	—	—
580	$-23.0 = 0.096$	$23.3 = 0.097$	—	—
610	$-21.0 = 0.096$	$21.5 = 0.098$	$42.6 = 0.097$	$42.5 = 0.097$

DISCUSSION OF RESULTS

The curves obtained by the use of filters 560 and 580 are quite similar, and it is evident that Beer's law was not followed. However, when filter 610 was used and the solution was placed in a 4 inch cell, a straight line was obtained, and except in the very low concentrations the same was true when a 1 inch or a 2 inch cell was used. Filter 610 gave considerable hue difference, which made the matching of the two halves of the photometric field somewhat difficult. No attempt was made to obviate this difficulty by interposing other filters in the light beam traversing the wedge of the photometer, as with little practice it is possible to obtain reproducible and accurate results. Filter 590 also offered some hue differences, especially in the more concentrated solutions. The fact that the hue differences with these two filters are so great in the higher concentrations indicates that the photometer readings are only apparent and not real, since the light transmission consistently favors the red. Therefore it is suggested that light filters centering around 560 and 580 mμ be used.

In the absence of a photometer, excellent results may be obtained when a set of standard methanol solutions are examined at the same time as the unknown, and 6 inch Nessler tubes are used instead of test tubes. In this case the solutions need not stand more than an hour.

The details of the method used after the sample had been distilled and diluted are as follows:

METHOD

REAGENTS

Potassium permanganate solution.—Dissolve 3 grams of $KMnO_4$ and 15 ml. of sirupy phosphoric acid (85%) in 100 ml. of distilled water.

Oxalic-sulfuric acid solution.—Dissolve 5 grams of $H_2C_2O_4$ in 100 ml. of H_2SO_4 (1 +1).

Modified Schiff's reagent.—Dissolve 0.2 gram of Kahlbaum's rosaniline HCl in about 120 ml. of hot water. Cool, and add 2 grams of Na_2SO_3 previously dissolved in 20 ml. of water. Add 2 ml. of concentrated HCl, dilute the solution to 200 ml., and place in the refrigerator for at least 24 hours before using.

Place 4.75 ml. of water and 0.25 ml. of the sample previously diluted to about 22% total alcohols by volume in a 6″ test tube, or in a 6″ Nessler tube if no photometer is used. Add 2 ml. of the $KMnO_4$ solution, mix thoroughly without inverting the tube, allow to stand 10 minutes with occasional shaking, and then add 2 ml. of the $H_2C_2O_4 - H_2SO_4$ solution. Add 5 ml. of the modified Schiff's reagent, mix thoroughly by inverting the tube three times, stopper, and allow to stand for about an hour if comparison is to be made in Nessler tubes, and $2\frac{1}{4}$ hours if a photometer is to be used, in which case not more than four determinations should be made at one time. This number may be made about every 20 minutes. After a standard curve has been made, the per cent of methanol may be read therefrom by applying the photometer readings in the usual manner.

CONCLUSIONS

The use of a neutral wedge photometer in connection with a curve made from a set of standards makes it possible to determine accurately very small amounts of methanol in distilled spirits, especially if all the conditions of temperature, age of reacting solutions, etc., previously mentioned, are strictly observed.

Inasmuch as the curves obtained by the use of the theoretical colored filters show that Beer's law was not being followed, it will be necessary for each analyst to construct his own standard curve.

For qualitative work the reagents may be older than stated and still give satisfactory results except when only a trace of methanol is present.

A NEW METHOD FOR READING THE COLOR OF WHISKEY

By G. F. BEYER (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.)

The method generally used for determining color of whiskey and other distilled spirits is the matching of the color with standard colored glass slides of the American Society of Brewing Chemists, Series No. 52, in a Lovibond tintometer. This process is quite difficult and unsatisfactory when the whiskey has acquired a considerable amount of color and when the sample varies slightly in tint or shade from the colored glass. Therefore the writer decided to utilize the neutral wedge photometer to test its usefulness for this purpose.

CHOICE OF PROPER FILTER

To determine the proper colored filter to use it was necessary to take a series of readings of a sample of whiskey with the photometer and to use colored glass filters covering the entire visible spectrum. An abridged spectrophotometric curve, Figure 1, obtained by this procedure shows that the greatest absorption occurs in the spectral region centering around 440 mμ. Theoretically, therefore, a colored filter transmitting light around

that particular wave length should be the proper one to use. However, after extended experimentation it was found that a filter centering around 460 mμ gave better results, and the curve, or rather a straight line, Figure 2, shows that Beer's law was being followed. The procedure follows:

ABRIDGED SPECTROGRAPH OF COLOR IN WHISKEY

FIGURE I.

% TRANSMISSION VS.

WAVE LENGTH $\frac{1}{2}$" CELL.
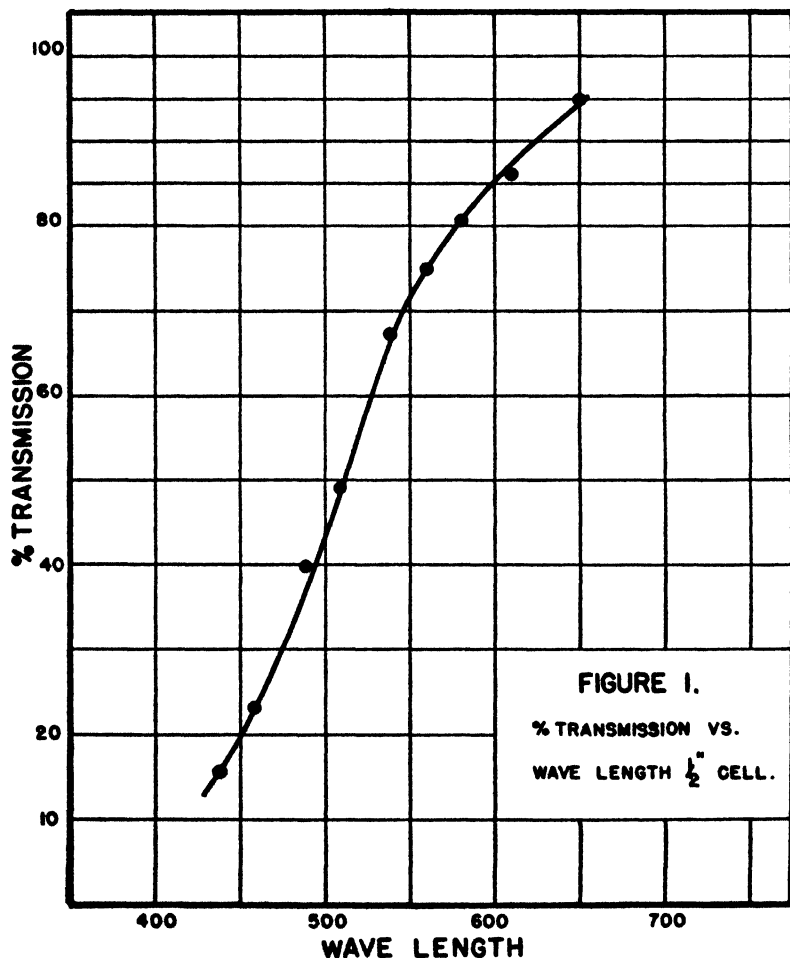
Adjust the zero point, using 50% ethyl alcohol in the $\frac{1}{2}$" cell. Use a sample of whiskey that has a reading of 15 in a Lovibond tintometer, when read in a $\frac{1}{2}$" cell, and take about 5 readings with the photometer. This gives one point on the curve. Obtain the other points in the curve by diluting this same whiskey with 50% alcohol and taking alternate readings in the tintometer and the photometer. Plotting the photometer readings against the tintometer readings gives a straight line that shows that the depth of color is directly proportional to the concentration. After

such a curve has been constructed, the color of any whiskey or distilled spirit may be obtained in terms of the Brewer's scale by taking readings in the photometer and applying them to the curve in the usual manner.

FIGURE II.

STANDARD CURVE OF COLOR
FOR WHISKEY FILTER 460.

PHOTOMETER READINGS.

NUMBERS ON GLASS SLIDES   BREWER'S SCALE.
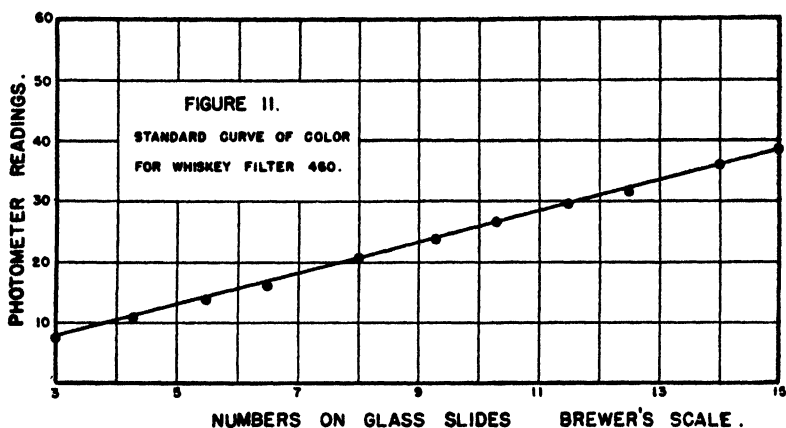
SUMMARY

The neutral wedge photometer affords a means of reading the color of whiskey and other distilled spirits with greater accuracy, greater ease of matching, and greater freedom from personal error than does the matching of colors in a comparator because it involves simply a brightness match in a monochromatic field, which eliminates color discrimination.

METHOD FOR THE DETERMINATION OF P-PHENYLENEDIAMINE AND P-TOLYLENEDIAMINE

By R. L. HERD (U. S. Food and Drug Administration, Buffalo, N. Y.)

Para-phenylenediamine has been used over a period of several years in various hair dye preparations. Its suitability for this purpose and the poisonous property of the diamines have created much interest in methods of identification. Although extensive reports have been published no convenient and reliable method has been offered for the quantitative estimation of this compound or of those compounds of similar constitution.

The method published by Callan[1] and Henderson depends upon the quantitative formation of benzoquinonedichlorimide. An excess of standard sodium hypochlorite solution is added and the solution titrated back with 0.1 N sodium arsenite solution, starch-iodide papers being used as indicator. This method is useful for the determination of the pure chemical individual, but may be unreliable in the presence of other materials that

[1] *J. Soc. Chem. Ind.*, **38**, 408–10 T (1919).

react with the hypochlorite solution. Since p-phenylenediamine is usually found in mixtures with other substances, such as starch, sulfur, and sulfites, a preliminary separation must be made before the method can be successfully used.

Preliminary investigation indicated that benzoquinonedichlorimide can be completely extracted with chloroform and that six atoms of iodine are liberated when the chloroform extract is shaken with a solution of potassium iodide and hydrochloric acid. Advantage was taken of these facts in developing the quantitative method presented here for the determination of p-phenylenediamine and p-tolylenediamine. The method is theoretically applicable to all para-diamines, excepting, perhaps, the acid diamines that would probably be held in the aqueous layer by the alkali. The method is also considered to be characteristic for the para-diamines with which hypochlorite forms a white precipitate, whereas various colored products are formed with the ortho and meta-diamines and p-diaminodiphenylamine. The purity of the p-phenylenediamine was determined by Kjeldahl nitrogen method, and the average per cent recovery, 99.74 (Table 1), was taken as the standard.

TABLE 1.—*Total nitrogen as p-phenylenediamine*

AMOUNT TAKEN	AMOUNT FOUND	RECOVERED
gram	*gram*	*per cent*
0.2704	0.2697	99.71
0.2613	0.2607	99.79
0.2478	0.2472	99.73
	Average	99.74

PROPOSED METHOD

Transfer 1 gram of the sample to a 200 cc. volumetric flask, dissolve in water, and make to the mark. By means of a pipet or buret add an aliquot of this solution representing 0.06–0.08 gram (smaller quantity may be used with good results) to a separator containing 5 cc. of a 5% alkaline NaOCl. If larger sample aliquots are used, repeat the operation, using more NaOCl or a smaller aliquot. (Insufficient NaOCl is indicated if the presence of a brown color is noted while the solution is being added.) Thoroughly mix the solutions during the addition of the aliquot by gently swirling the separator. After the charge has been added, stopper the separator and shake for about 10 seconds. Add 10 cc. of a 10% Na_2HAsO_3 solution, stopper the separator, and shake again. Extract the benzoquinonedichlorimide with two successive 25 cc. portions of $CHCl_3$ and combine the extracts in a second separator. Wash the combined extracts with 10 cc. of water and filter through a pledget of cotton into an iodine flask. Make an additional extraction, wash with the water, and combine with the major portion. Add 50 cc. of water containing 1 gram of KI and 3 cc. of HCl to the combined $CHCl_3$ extracts, stopper the flask, and shake vigorously for 1 minute. Titrate the liberated I with 0.1 N $Na_2S_2O_3$. Stopper the flask and shake vigorously at intervals during the titration. The I in the $CHCl_3$ acts as an indicator. Toward the end of the titration add starch solution for the final end point. Each cc. of 0.1 N $Na_2S_2O_3$ =0.001801 gram of p-phenylenediamine or 0.002035 gram of p-tolylenediamine.

Table 2 shows the percentage recovery of *p*-phenylenediamine and *p*-tolylenediamine based upon the proposed method.

TABLE 2.—*Recovery with proposed method*

AMOUNT TAKEN	AMOUNT FOUND	RECOVERED
	P-Phenylenediamine	
gram	*gram*	*per cent*
0.0119	0.0117	98.32
0.0133	0.0137	103.01
0.0140	0.0142	101.43
0.0239	0.0237	99.16
0.0265	0.0264	99.62
0.0279	0.0285	102.15
0.0298	0.0296	99.33
0.0358	0.0356	99.44
0.0384	0.0382	99.48
0.0528	0.0528	100.00
0.0597	0.0591	99.00
0.0609	0.0605	99.34
0.0619	0.0619	100.00
0.0673	0.0672	99.85
0.0674	0.0672	99.70
0.0713	0.0712	99.86
0.0726	0.0726	100.00
0.0746	0.0739	99.06
0.0793	0.0793	100.00
0.0896	0.0888	99.10
0.0900	0.0894	99.33
	Tolylenediamine	
0.0188	0.0184	97.87
0.0313	0.0311	99.36
0.0376	0.0373	99.20
0.0563	0.0558	99.11
0.0626	0.0620	99.04
0.0751	0.0744	99.07
0.0782	0.0776	99.23
0.0939	0.0932	99.25

The quinonedichlorimide may be used as an aid in establishing the identity of the p-diamine.

Proceed as follows:

Dissolve in 10 cc. of water, sufficient material to represent about 0.5 gram of the diamine and transfer to a separator. Extract with two successive 25 cc. portions of petroleum ether and add the aqueous portion to a second separator containing 25 cc. of NaOCl as directed in the method. Extract the dichloramide with about 10 cc. of petroleum ether, and wash the ether layer with about 10 cc. of water. Filter the extract through cotton into a 50 cc. beaker. Evaporate the ether with the aid of gentle heat and dry the residue in a vacuum desiccator. Accurately weigh about 0.1 gram of the dichloramide and titrate as directed previously. From the number of

cc. of 0.1 N Na$_2$S$_2$O$_3$ consumed and weight of sample, calculate the molecular weight of the dichloramide and compare with the theoretical.

$$\text{M.W.} = \frac{60000 \times \text{Wt. sample}}{\text{cc.Na}_2\text{S}_2\text{O}_3}$$

Some results are given in Table 3 for benzoquinonedichloramide, toluoquinonedichloramide and an unknown purported to contain *p*-phenylenediamine.

TABLE 3.—*Molecular weight*

BENZOQUINONEDICHLORAMIDE	FOUND	THEORETICAL
gram		
0.1294	174.63	174.96
0.1811	174.33	
TOLUOQUINONEDICHLORAMIDE		
gram		
0.1332	188.31	188.98
0.1496	187.94	
DICHLORAMIDE FROM SAMPLE OF HAIR DYE		
gram		
0.0335	173.88	

DISCUSSION OF METHOD AND RESULTS

Directions are given for the use of an aliquot representing 0.06–0.08 gram of the diamine, since this is the optimum amount. The analyst may vary the preparation and size of sample without materially changing the results.

It was found that the chloroform extract from the hypochlorite liberated a small amount of iodine when shaken with a solution of potassium iodide, and for this reason the sodium hypochlorite was destroyed with sodium arsenite subsequent to extraction. Benzoquinone does not react with sodium arsenite in alkaline solution or with potassium iodide in neutral solution.

Some of the results are somewhat high, which is probably due largely to over titration on the small-sized sample. The results compare quite favorably with those of the nitrogen determination.

CONCLUSIONS

A simplified and accurate method for the quantitative determination of *p*-phenylenediamine and *p*-tolylenediamine is presented. The method is specific for these products since their identity may be established by molecular weight tests.

FILTERING BEFORE ADDITION OF PLATINIC CHLORIDE IN THE ANALYSIS OF FERTILIZERS FOR POTASH*

By H. R. ALLEN (Kentucky Agricultural Experiment
Station, Lexington, Ky.)

In the two previous papers,[1,2] the presence of considerable insoluble residue in the potassium chloroplatinate in the potash determination in fertilizers was noted. Less residue and more potash were found if the ignition was conducted at higher temperatures, except in the case of a 15 minute ignition in the electric muffle, when silica dishes were used. Ignition in platinum dishes gave a smaller residue and larger potash content than was obtained in silica dishes.

Thornton and Kraybill[3] obtained appreciable amounts of insoluble residue in the potassium chloroplatinate when the latter was not allowed to stand in the acid alcohol for 15 minutes, but otherwise negligible amounts of residue were obtained. They found the residue consisted chiefly of silica, iron, aluminum, and magnesium phosphates. At the 1937 meeting of this Association the Associate Referee on Potash reported results[4] on filtering the solution before the addition of the platinic chloride, in which case lower results for potash were obtained when the solution was filtered, indicating the presence of an insoluble residue.

This paper gives results for insoluble residue obtained in 360 routine potash determinations made in groups of 60 samples each, in which the solution was filtered before the addition of the platinic chloride. The residue was concentrated by filtering five determinations through the same ashless filter paper (C. S. & S. No. 589, blue ribbon) and ashing the papers in groups of 4. The ignited residue was treated with 20 cc. of 80 per cent alcohol, containing 10 per cent by volume of concentrated hydrochloric acid and allowed to stand for 2 hours under a bell jar, then transferred to a filter paper and washed with ammonium chloride and alcohol. The insoluble residue was analyzed for silica, iron and aluminum oxides, phosphoric acid, and in one case for sodium. The residue from one group was analyzed for potassium. Ignitions were made in both silica and platinum dishes, as indicated. The effect of the addition of four drops of hydrochloric acid (1+1) in the dish after ignition and before the addition of any water was investigated.

The official method for potash[5] was used with the exceptions noted. One drop of tributyl citrate was added to the flask before the ammonium oxa-

late digestion. This prevented foaming. Final ignition over the Fisher burner as previously reported[2] was used. A platinum foil was placed lightly over the silica dishes during the final stage of the ignition to partially compensate for the difference in heat conductance of the platinum and silica. When the filtering was done before addition of the platinum solution, hot water was added to the dish several times and decanted through the filter, time being allowed for complete solution. If necessary, the residue was loosened or broken up with a solid glass rod or rubber policeman. After each filtration, the filter paper was washed well with hot water. After addition of platinum solution and evaporation to dryness 6 cc. of 95 per cent alcohol, to which had been added 10 per cent by volume of hydrochloric acid (sp. gr. 1.18) was placed in each dish and rubbed well with a policeman, and a minimum of 15 minutes was allowed to elapse before filtering. The potassium chloroplatinate of the succeeding set of determinations was transferred to the same crucibles without washing out the potash salt of the preceding set. After five analyses in each crucible the potassium chloroplatinate was leached out with boiling water, the crucibles were dried and weighed, and the increase in weight was noted.

After sixty potash determinations had been made in twelve crucibles the potassium chloroplatinate in six crucibles was rewashed six times with 3 cc. portions of 80 per cent alcohol, and the potassium chloroplatinate in the other crucibles was washed with equal amounts of 95 per cent alcohol. The average loss in weight per crucible when 80 per cent alcohol was used was 1.46 mg.; the average loss in weight when 95 per cent alcohol was used was 0.25 mg. Previous to this washing this group had been well washed with 95 per cent alcohol, then with 80 per cent alcohol, to eliminate all traces of the ammonium chloride washing. In view of the above results, determinations reported in this paper were washed with 95 per cent alcohol, and 95 per cent alcohol was used in preparing the acid alcohol except for the digestion of the residue concentrates, when 80 per cent alcohol was used.

DISCUSSION OF RESULTS

Larger residues were obtained when the ignition was conducted in silica dishes, as is shown in Table 1. The average residue per determination in silica was 2.27 mg., in platinum 1.51 mg. Contact with acid alcohol for 2 hours dissolved appreciably less if the ignition was conducted in silica than in platinum, the average losses being 0.26 and 0.70 mg., respectively. Addition of hydrochloric acid to the dishes after ignition and before water was introduced, reduced the insoluble residue somewhat and aided materially in loosening the material from the dish and in solution. Addition of hydrochloric acid at this stage did not increase the residue left in the filter crucibles. The percentage of potassium oxide equivalent to the insoluble residues is given in Table 2.

TABLE 1.—*Insoluble residues obtained by filtering before addition of platinic chloride*
(Results expressed as mg.)

	GROUP NUMBER (60 ANALYSES)	SILICA DISH. HCl IN DISH[a] (30 ANALYSES)	SILICA DISH. NO ACID. (30 ANALYSES)	PLATINUM DISH. HCl IN DISH[a] (20 ANALYSES)	PLATINUM DISH. NO ACID. (30 ANALYSES)
Residue after ignition	1	15.9	28.8		29.8
	2	12.1	18.8	2.6	
	3[b]	52.2	65.0		26.4
	4[c]	79.6[d,c]	58.1		19.7
	5	43.9[d]	80.8[a]	12.4	
	6	26.4[f,d]	40.1[a,d]		
Residue insoluble in acid alcohol	1	12.5	23.9		26.0
	2	8.5	14.6	1.2	
	3	46.7	57.0		9.4
	4	72.0	53.3		9.9
	5	40.0	75.2	10.2	
	6	22.8	36.6		
Average residue per determination on filter pads after 5 analyses in each crucible[g]	1	0.44	0.44		0.45
	2	0.30	0.40	0.05	
	3	0.46	0.85		0.11
	4	0.61	0.78		0.32
	5	0.64	0.82	0.68	
	6	0.42	0.51		

[a] 4 drops HCl (1+1) in each dish before addition of water.
[b] Residues in this group in contact with acid alcohol 1 hour.
[c] Tributyl citrate not added in this group.
[d] New silica dishes used.
[e] No HCl in dish before water.
[f] Platinum foil not used over dishes during ignition.
[g] Platinum was not deposited in the residues.

TABLE 2.—*Average per cent K_2O equivalent to insoluble residues.*
Basis 0.25 gram sample

	GROUP NUMBER	SILICA DISH	SILICA DISH	PLATINUM DISH
Residue insoluble in acid alcohol	1	0.05	0.09	0.10
	2	0.03	0.06	0.005
	3	0.18	0.22	0.04
	4	0.28	0.21	0.04
	5	0.15	0.29	0.04
	6	0.09	0.14	
Residue left in filter crucibles	1	0.03	0.03	0.03
	2	0.02	0.03	0.004
	3	0.04	0.07	0.01
	4	0.05	0.06	0.02
	5	0.05	0.06	0.05
	6	0.03	0.04	

The average residue in per cent potassium oxide equivalent per determination was 0.14 for silica dishes to which hydrochloric acid was added first and 0.18 if hydrochloric acid was not added; 0.02 for platinum dishes to which hydrochloric acid was added and 0.06 if hydrochloric acid was not added.

TABLE 3.—*Analysis of insoluble residues*
(Results expressed as mg.)

GROUP NUMBER		SILICA DISH. HCl IN DISH	SILICA DISH. NO ACID	PLATINUM DISH. HCl IN DISH	PLATINUM DISH. NO ACID
1	SiO_2	9.8	8.0		7.0
	$Fe_2O_3+Al_2O_3$	1.0	4.3		5.5
	P_2O_5	0.7	8.2		9.4
	Undetermined	1.0	3.4		4.1
2	SiO_2	2.6	2.1	0.4	
	$Fe_2O_3+Al_2O_3$	1.9	2.4	0.3	
	P_2O_5	2.6	3.8	0.3	
	Undetermined	1.4	6.3	0.2	
3	SiO_2	2.0	5.3		0.4
	$Fe_2O_3+Al_2O_3$	11.3	14.6		2.2
	P_2O_5	21.6	25.1		4.0
	Undetermined	11.8	12.0		2.8
4	SiO_2	3.0	4.4		1.3
5	SiO_2	0.6	1.3	0.1	
	$Fe_2O_3+Al_2O_3$	11.4	23.2	4.1	
	P_2O_5	20.4	38.7	5.0	
	Na[a]	0.8	1.3	0.4	
	Undetermined	6.8	10.7	0.6	
6	SiO_2	1.2	1.9		
	K_2O	2.4[b]	3.4[c]		

[a] Determined by uranyl acetate method.
[b] Equivalent to average of 0.05% K_2O per determination.
[c] Equivalent to average of 0.067% K_2O per determination.

Table 3 gives a partial analysis of the residues and shows the chief components to be silica, iron and alumina, and phosphorus. Magnesium was found in qualitative tests in very small amounts in some instances. Calcium was not found. Potassium was found by means of the spectroscope. All analyses in Group 6 were made in silica dishes. Two of the residues in this group were analyzed for potassium and an amount found equivalent to an average per determination of 0.05 and 0.067 per cent potassium oxide.

Sodium was found in appreciable quantity in Group 5, but was not determined prior to this group. The larger size of the residues found in silica dishes as compared with platinum was due only in small part to silica.

The ignition in platinum was at considerably higher temperature than in silica due to the difference in heat conductance. The residue was always fused in platinum dishes, but this was not the case in silica. Since larger residues were obtained in silica dishes without a corresponding increase in silica, a probable explanation seems to be the formation of sodium metaphosphate, which is relatively insoluble, at the lower temperature. At the higher temperature obtained in platinum, this is changed to sodium pyrophosphate, which is soluble.

$$4NaPO_3 - P_2O_5 = Na_4P_2O_7.$$

If the corresponding potassium salts were used, this explanation would account for the larger potassium content and smaller residue obtained when platinum dishes were ignited at the higher temperature, as reported in the previous paper.[2] In the early work of this Association, Scovell, Peter, and Curtis[1] found it necessary to heat the ignited residue for a sufficient time with water and hydrochloric acid to dissolve any potassium metaphosphate formed. Solutions of samples requiring duplication were not filtered before platinic chloride was added, but the potash content was determined by leaching out the potassium chloroplatinate and reweighing the crucible. Table 4 gives the average increase or decrease in per cent potassium oxide obtained by this method over the filtering process. The results are not conclusive for silica dishes, but show an increase in potassium oxide when platinum dishes were used and the potassium chloroplatinate leached out.

TABLE 4.—*Average increase or decrease in per cent K_2O obtained when K_2PtCl_6 was leached out and crucible reweighed over K_2O from solutions filtered before addition of platinic chloride. Basis 0.25 mg. sample*

GROUP NUMBER	NUMBER OF ANALYSES AVERAGED	SILICA DISHES	NUMBER OF ANALYSES AVERAGED	PLATINUM DISHES
1	9	−0.11	4	−0.02
2	4	+0.04	4	+0.03
3	8	−0.09	8	+0.02
5	5	+0.02	4	+0.11
6	5	+0.09	6	+0.10
Average	31	−0.03	26	+0.048

SUMMARY

Ignition in a silica dish gave a larger residue, less soluble in acid alcohol, than did ignition in platinum. Filtering the residue out before platinic chloride was added gave slightly less potash, and potash was sometimes found in the material removed. The amount of residue filtered from ignition in silica, if counted as potassium oxide, would increase the potash

[1] *Proceedings of the 13th Annual Convention of the A.O.A.C.*, Bull. 49, 42.

content erroneously, in some instances. Residues obtained after ignition in platinum were small, particularly when hydrochloric acid was added when the ignited material was dissolved in the dish. Ignition in platinum, solution of the ignited salts in hydrochloric acid and water, and determination of potassium chloroplatinate by weighing the filter, crucible and contents, leaching out and weighing the crucible, gave the most potassium oxide. One drop of tributyl citrate in the digestion prevented foaming.

IMPROVED MOLYBDENUM BLUE REAGENTS FOR DETERMINATION OF PHOSPHORUS AND ARSENIC

By JOHN A. SCHRICKER and PAUL R. DAWSON (Soil Fertility Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.)

Investigations in these laboratories, relating to soil phosphorus and the efficiency of its utilization by crop plants, have necessitated refinement in methods for the quantitative determination of minute concentrations of phosphoric acid in soil extracts and plant materials by the colorimetric ceruleomolybdate procedure. When Zinzadze's (40) molybdenum blue reagent was used for this purpose, off colors and interference effects were encountered, which were later traced to limitations in the applicability of the reagent under the conditions imposed. These difficulties were minimized by modification of the reagent and the conditions of its reaction with phosphoric acid; and the reliability and range of application of the method were greatly improved.

The name "molybdenum blue" is properly applied to that complex oxide or series of oxides of molybdenum lying between Mo_2O_5 and MoO_3. Its relative stability in combination with certain substances (phosphoric, arsenic, and silicic acids) and its instability in their absence have led to its wide use in colorimetry. Bucholz (12, 13) is credited with its discovery in 1803 when he triturated molybdic oxide and molybdenum metal together in water suspension. Subsequent investigations (1, 2, 3, 7, 8, 9, 10, 17, 18, 21, 22, 23, 25, 26, 28, 30, 34, 37) of its composition have yielded a variety of data and opinions. However, whether molybdenum blue is a single oxide or a series of oxides has not been conclusively determined, nor does the evidence justify the assignment of a more specific constitution than is expressed by the empirical formulas Mo_nO_{3n-1} for the unstable anhydrous oxide and $(Mo_nO_{3n-1})2H_3PO_4$ for its stable phosphoric acid complex. The opinion held by many workers, that the complex contains pentavalent molybdenum, is substantiated by considerable corroborative evidence.

In 1887 Osmond (29) applied molybdenum blue to the colorimetric determination of phosphorus by reduction of phosphomolybdate with stannous chloride. His method, with variations, has been used almost universally since, notably by Denigés (15, 16) and Truog and Meyer (31). Although Denigés called the blue complex thus formed "stable molybdenum blue," its stability is relative and fleeting. Further search has been necessary in the direction of different reducing agents and modified conditions to achieve truly stable colors. Bell and Doisy, (4), Briggs (11), Benedict (5), Fiske and Subbarow (20), and Tschopp and Tschopp (32) made excellent contributions in the application of organic reducing agents.

All the procedures mentioned are based on the formation of the blue complex by the assumed specific reduction of phosphomolybdate, but Berenblum and Chain (6) have recently shown that molybdic acid is also reduced to a degree dependent upon sulfuric acid concentration and reducing agent strength. These two investigators have already surveyed the problem toward which this study is an independent approach. They solve the problem of reagent concentration by the unique method of extracting the phosphomolybdate with isobutyl alcohol followed by shaking with stannous chloride solution to obtain the pure blue complex in alcoholic solution free of excess reagents.

A departure was made by Zinzadze (36, 37, 38, 39, 40) in developing the reagent upon which the work reported here is based. He first prepared "unstable" molybdenum blue and then permitted it to react in excess with the phosphoric acid. This method resulted in the formation of really stable blue complexes, the excess molybdenum blue oxide being destroyed under proper experimental conditions. Zinzadze's reagent was prepared by the reduction of a sulfuric acid solution of molybdic oxide with powdered molybdenum metal. It was carefully standardized with respect to reduction and acidity to conform to the composition: 0.04 M (0.08 N) $MoO_2 \cdot 4 \ MoO_3$ in 19 N sulfuric acid. Later the values were increased to approximate 0.05 M $MoO_2 \cdot 4.9 MoO_3$ in 25 N sulfuric acid. This reagent can be preserved indefinitely in a glass-stoppered bottle. In the determination of phosphorus, the test solution is digested for one hour on the steam bath with acidified sodium bisulfite to prevent interference from iron, arsenic, and nitrates. A measured quantity of the molybdenum blue reagent is added and heating continued 30 minutes longer to develop the color. Probably this time factor, the involved procedure for preparing the reagent, and the extreme precautions advised have diverted much deserved attention from Zinzadze's method.

In the modified method described here, Zinzadze's reagent and procedure have been simplified, and certain deficiencies of the original reagent for determination of both phosphorus and arsenic have been corrected. There is also described an alternative method, combining desirable features of the methods of Zinzadze and of Tschopp and Tschopp.

EXPERIMENTAL

When the Zinzadze method was applied to the determination of phosphoric acid in buffered soil extracts and similar solutions, difficulty was experienced, in many instances, with yellow tints that made visual comparison of the colors unreliable. Fading or incomplete development of color, poor duplication of results, and excessive "blanks" were other difficulties encountered particularly in the case of acetic acid-sodium acetate solutions of the type rather widely used as a buffered extractant for soil phosphorus. Apparently these difficulties were associated with excessive yellow pentavalent molybdenum and with the salt or buffer effect of the extractant, as well as of the sodium bisulfite added. Preliminary experiments showed that the interference of pentavalent molybdenum could be minimized, without decreasing the efficiency of the reagent, by lowering the reduction concentration (Mo_2O_5)* from 0.1 to 0.04 N.

It was also found that the interference from foreign salts could be decreased by the substitution of an equivalent quantity of sulfurous acid for the sodium bisulfite and sulfuric acid as used in the Zinzadze procedure and also further reduced by preliminary adjustment of the pH of the test solution to the end point of quinaldine red rather than to that of beta-dinitrophenol (original method). However, minimum interference was attained only by increasing the sulfuric acid concentration of the reagent itself from 25 to 36 N.

This effect of acidity was found to be associated with and limited by the molybdic oxide concentration,† varying inversely with the latter. In other words, the critical factor is the sulfuric acid-molybdic oxide ratio rather than their independent concentrations. This finding merely extends the observations of Zinzadze (*loc. cit.*) on silica interference to include salt effect. With these data as a guide, and modifying the Zinzadze procedure to substitute sulfurous acid and quinaldine red, as indicated above, the writers made experiments to determine the concentrations of molybdic oxide, sulfuric acid, and the reduction that would give maximum color development with minimum interference.

Three series of reagents, in which these factors varied in turn, were applied to pure aliquots representing 20 gamma (0.02 mg.) of phosphorus and 50 gamma of arsenic, respectively. The resulting color intensities in the test solutions at a uniform volume of 50 ml. were measured in a compensating photoelectric colorimeter designed and constructed in this laboratory by the senior author. Single readings were taken of single determinations, and no results were discarded. The results are given in Tables 1, 2, and 3. The concentrations of the reagent constituents are expressed on the basis of the diluted test solutions. Since the reagents

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* The term "reduction concentration" applies to the concentration of pentavalent molybdenum oxide (Mo_2O_5) resulting from reduction of the hexavalent molybdenum oxide (MoO_3).
† The term "molybdic oxide concentration" is restricted to the concentration of hexavalent molybdenum oxide (MoO_3) remaining after reduction.

were added in the proportion of 0.5 ml. per 50 ml. of final volume of test solution, these concentrations represent .01 of those in the original reagents. An exception occurs in the case of Table 2, where supplementary additions of acid were necessary in order to exceed 0.36 N, the maximum attainable by the 100-fold dilution of the concentrated reagent. The corresponding color intensities are expressed in terms of the photometer transmission readings.

TABLE 1.—*Effect of molybdic oxide concentration on intensity of color from 20 gamma of phosphorus and 50 gamma of arsenic at a uniform reduction concentration of 0.0004 N and sulfuric acid concentration of 0.36 N*

| MOLAR CONCENTRATION OF MOLYBDIC OXIDE | PHOTOMETER READINGS | | | |
| | 30 MINUTE DEVELOPMENT | | 60 MINUTE DEVELOPMENT | |
	PHOSPHORUS	ARSENIC	PHOSPHORUS	ARSENIC
0.0010	—	—	94.8	96.7
0.0012	—	—	85.2	90.5
0.0014	—	—	61.4	80.1
0.0016	89.5	89.5	61.3	73.5
0.0018	59.6	82.4	58.5	64.8
0.0020	59.5	73.2	58.7	61.0
0.0022	59.2	62.3	59.0	60.5
0.0024	60.5	63.7	59.0	60.5
0.0026	60.0	61.9	58.4	60.1
0.0028	59.6	61.2	59.1	60.5
0.0030	59.0	61.2	58.7	60.6
0.0032	59.5	59.5	58.2	60.4

The data in Table 1 show that the concentration of molybdic oxide, with uniform sulfuric acid and reduction concentrations of 0.36 N and 0.0004 N, respectively, may be varied from 0.0018 M to 0.0032 M for phosphorus and from 0.0026 to 0.0032 M for arsenic without affecting the transmission value (color intensity), for a 30 minute period of color development. Increasing the period of color development to 60 minutes had no appreciable effect on the color intensity for either phosphorus or arsenic, but extended the range of uniform color development for arsenic down to 0.0020 M molybdic oxide. Below 0.0018 M MoO$_3$ the phosphomolybdenum blue drops off sharply, and for arsenomolybdenum blue there is a gradual decline below 0.0026 M MoO$_3$, after 30 minutes and below 0.0020 after 60 minutes.

In Table 2 are presented the effects on color intensity of varying the concentrations of sulfuric acid at a uniform reduction concentration of 0.0004 N and molybdic oxide concentrations of 0.0018 and 0.0032 M for phosphorus and arsenic, respectively. The molybdic oxide concentration for phosphorus was the lowest one shown above to yield complete color

TABLE 2.—*Effect of sulfuric acid concentration on intensity of color from 20 gamma of phosphorus and 50 gamma of arsenic at a uniform reduction concentration of 0.0004 N and molybdic oxide concentrations of 0.0018 M for phosphorus and 0.0032 M for arsenic*

NORMALITY OF SULFURIC ACID	pH*	PHOTOMETER READINGS	
		PHOSPHORUS	ARSENIC
0.24	0.83	58.2	58.2
0.27	0.80	58.0	59.0
0.30	0.77	58.2	59.6
0.33	0.74	58.4	59.0
0.36	0.70	58.0	59.5
0.39	0.68	61.2	59.6
0.42	0.66	67.3	59.4
0.45	0.64	75.6	62.2

* pH determinations by glass electrode.

development with a sulfuric acid concentration of 0.36 N, and was selected in order to maintain the maximum ratio of sulfuric acid to molybdic oxide necessary for minimum interference by silica and salts. These considerations could be ignored in the case of arsenic, since this element is usually separated (14, 24, 27, 33, 35) by distillation or precipitation prior to its determination. The maximum molybdic oxide value was therefore chosen as it gave the most rapid development of color.

The results show that with pure solutions changes in sulfuric acid concentration from 0.24 N to 0.36 N (the maximum attainable by direct

TABLE 3.—*Effect of reduction concentration on intensity of color from 20 gamma of phosphorus and 50 gamma of arsenic at a uniform sulfuric acid concentration of 0.36 N and molybdic oxide concentration of 0.002 M*

NORMALITY OF REDUCTION CONCENTRATION	PHOTOMETER READINGS	
	PHOSPHORUS	ARSENIC
0.00040	58.0	59.5
0.00044	57.0	62.3
0.00050	57.8	62.5
0.00058	57.2	62.3
0.00066	57.8	62.5
0.00080	57.2	61.5
0.00100	57.3	60.2*
0.00134	59.6	60.4*
0.00200	77.9	60.1*
0.00200	57.0†	—
0.00400	No color‡	

* Practically complete development of color due undoubtedly to compensatory oxidation of Mo₄O₇, not so rapid in case of phosphorus because of presence of sulfurous acid.
† After 60 minutes on the steam bath.
‡ The composition of this reagent conforms to the formula Mo₄O₇: an excess of MoO₃ over this composition is necessary to produce color.

addition of the reagent in the proportion used) are without effect on the color intensity. The *p*H of the solutions at these concentrations is well below 1.0, the value above which blue color tends to appear in the absence of phosphorus or arsenic.

The data in Table 3 show that for the 30-minute period of color development used, variation in the reduction concentration from 0.00040 *N* to 0.0010 *N* was without material effect on the color intensity for phosphorus. Increasing the period of color development to 60 minutes extended the range of uniform color intensity to 0.00200 *N* reduction. For arsenic the color intensity did not vary materially over the entire range even with 30 minutes' color development.

The results of these experiments indicate that within certain limits and with pure solutions color intensity is comparatively independent of the molybdic oxide, sulfuric acid, and reduction concentrations. However, for maximum color development, with minimum interference from yellow tints, silica, and salt effects, the choice of conditions is narrowed.

While the intensity of the blue colors remains relatively uniform over a considerable range of reduction concentrations, interference from the yellow tints due to excess of pentavalent molybdenum increases with the degree of reduction. For example, the blue colors developed at 0.0004 *N* and 0.002 *N* are identical in intensity when compared visually through a blue filter or in an electrophotometer as in the above experiments. In white light, however, the yellow interference at the higher reduction greatly increases the uncertainty of visual comparison.

Similarly, while pure phosphate solutions yield colors of relatively uniform intensity despite considerable variation in the concentration of molybdic oxide and sulfuric acid, interferences arise in the presence of extraneous substances unless the range of variation in these concentrations is restricted. As shown by Zinzadze (*loc. cit.*) the interference of silica is minimized by increasing the ratio of sulfuric acid to molybdic oxide. Experiments preliminary to this investigation and the data presented later show that similar conditions apply to interference by salts. The sulfuric acid concentrations must be sufficient to hold the *p*H below 1.0, despite the presence of salts of appreciable buffer capacity.

Accordingly, the conditions for minimum interference of yellow tints, silica, and salt effects require the minimum reduction and molybdic oxide concentrations and maximum sulfuric acid concentrations compatible with uniform and complete color development. As shown by the above experimental data these conditions are most nearly approached for phosphorus with molybdic oxide, sulfuric acid, and reduction concentrations of 0.0018 *M*, 0.36 *N*, and 0.0004 *N*, respectively, in the test solution. For arsenic, a higher molybdic oxide concentration, namely 0.0032 *M*, is necessary for full color development.

To attain these conditions in the diluted test solution the concentrated

reagent for phosphorus would have a molybdic oxide concentration of 0.18 M, a sulfuric acid concentration of 36 N, and a reduction concentration of 0.040 N. For arsenic the sulfuric acid and reduction concentrations would remain the same, but the molybdic oxide concentration would be raised to 0.32 M. These reagents are therefore concentrated sulfuric acid solutions of 0.02 M $Mo_{10}O_{29}$ for phosphorus and 0.02 M $Mo_{17}O_{50}$ for arsenic. The improved reagents and procedures for their application to determination of phosphorus and arsenic, as described later, were developed in conformity with these findings.

Incidental experiments were conducted with the application of unreduced sulfomolybdic acid reagents equivalent in concentrations to the improved molybdenum blue reagent to determinations of phosphorus and arsenic by procedures involving use of organic reducing agents. Of the three organic reducing agents in common use, namely hydroquinone, amino naptholsulfonic acid, and methylparamidophenol sulfate (metol), the last was found to be most satisfactory. Color development was complete in 50 minutes at 95° C. with phosphorus, but though proportionate was only 90 per cent complete with arsenic. An alternative method, based on these trials and combining desirable features of the methods of Zinzadze (*loc. cit.*) and Tschopp and Tschopp (32) is included below.

In connection with the preparation of the reagents, it was noted that molybdic oxide dissolves more rapidly in hot concentrated sulfuric acid than in 25 N acid; that the reduction of the molybdic oxide by powdered molybdenum in this solution is practically immediate and quantitative at about 140–150° C.; and that in the presence of an excess of molybdenum metal a final composition corresponding to Mo_2O_5 is attained. Thus two avenues are offered by which a reagent of definite composition may be prepared: (1) By reducing sulfomolybdic acid at 150° C. with a calculated quantity of molybdenum metal; (2) the same reduction with an excess of metal followed by the mixing of the proper quantity of unreduced solution with reduced solution decanted from the excess metal as described by Zinzadze.

The several procedures finally developed for preparation of the improved reagents and for their application to the determination of phosphorus and arsenic are as follows:

METHOD A, PHOSPHORUS

REAGENTS

(a) *Molybdenum blue for phosphorus.*—Ignite a portion of C.P. MoO_3 in a porcelain dish at dull red heat and below the melting point in a muffle for about 1 hour. Cool, and weigh 6.96 grams into a 500 ml. Kjeldahl flask. Add 250 ml. of concentrated (36 N) H_2SO_4 and a few glass beads, and boil the mixture gently until solution is complete (a slight cloudiness does not matter).

Cool to about 150° C. and add 0.16 gram of C.P. powdered Mo metal on a small watch-glass slid carefully down the neck of the flask so that all of the metal is introduced. Rotate the flask occasionally for 10 minutes, when the metal should be

entirely dissolved, otherwise it can be readily observed against the background of the deep green solution when the flask is held above the level of the eyes. If there is a residue of metal, reheat the solution to 150° C. When the metal is entirely dissolved, cool the solution, dilute a convenient aliquot (5 or 10 ml.) with about 5 volumes of water and titrate with 0.1 N or 0.02 N KMnO$_4$ until the appearance of a pink color lasting for 1 minute. From this titration calculate the reduction concentration of the solution which should be approximately 0.04 N. If less than 0.036 N, add a calculated quantity of Mo sufficient to bring the reagent up to 0.04 N and reheat to 150° C. Cool, recheck the titer, and preserve the bright blue solution in a glass-stoppered bottle.

(b) *Standard phosphate.*—Prepare aqueous monopotassium phosphate solutions equivalent to 1, 10, 100, etc., gamma of phosphorus per ml.

(c) *Sulfurous acid.*—Saturate 500 ml. of water with SO$_2$ gas. In cases where NaHSO$_3$ may be used the following is recommended.

(d) *Sodium bisulfite.*—Dissolve 5.2 grams of C.P. NaHSO$_3$ in 100 ml. of normal H$_2$SO$_4$. Prepare the solution weekly and keep stoppered.

(e) *Sulfuric acid.*—5% aqueous solution.

(f) *Sodium carbonate.*—10% aqueous solution.

(g) *Quinaldine red.*—0.01% aqueous solution.

<div align="center">DETERMINATION</div>

Pipet an aliquot of the solution containing phosphorus into a 50 ml. volumetric flask. Add one drop of the quinaldine red and adjust to the point where the red color just disappears with the dilute Na$_2$CO$_3$ or H$_2$SO$_4$. Add water to a volume of approximately 25 ml., then 5 ml. of either the sulfurous acid or NaHSO$_3$ solution. Digest on the steam bath for 30 minutes or for 20 minutes after the temperature has reached 95° C. (If the aliquot is comparatively free of arsenic and nitrate (no more than 50 gamma) this time may be shortened to 10 minutes.) Carefully pipet 0.5 ml. of the molybdenum blue reagent into the flask, letting it run down the side of the neck to avoid sputtering. Wash down the neck of the flask with two or three drops of water, mix, and continue digestion for 30 minutes more. Cool, make to 50 ml., mix, and read against a standard similarly prepared, or, preferably, in a photoelectric colorimeter.

It is desirable to read the colors within 4 hours as there is a 2–5% fading during the first 24 hours. However, this is not sufficient to preclude the reading, by ordinary colorimetric means, of colors left overnight, as such fading is detectable only by a photometer. After 24 hours, if the flasks are stoppered, the colors remain quite constant for a week or more, even when exposed to light.

<div align="center">METHOD B, PHOSPHORUS</div>

<div align="center">REAGENTS</div>

(a) *Sulfomolybdic acid.*—Dissolve 7.2 grams of ignited C.P. molybdic oxide in 250 ml. of concentrated H$_2$SO$_4$ as in Method A, first paragraph. Cool, and preserve the solution in a glass-stoppered bottle.

(b) *Metol.*—Dissolve 0.42 gram of methylparamidophenol sulfate and 6.3 grams of Na$_2$SO$_3$ in water and make to 100 ml.

Other reagents.—See Method A.

<div align="center">DETERMINATION</div>

Proceed as directed in Method A up to the point: "Carefully pipet 0.5 ml. of the molybdenum blue reagent," etc. Instead, pipet 0.5 ml. of the sulfomolybdic acid reagent and 1.0 ml. of the metol reagent into the flask, observing the same precautions. Continue the digestion for 30 minutes on the steam bath, cool, make to volume, mix, and read as directed in Method A.

In the comparative absence of arsenic and nitrate the preliminary digestion may be shortened, as in Method A.

METHOD C, ARSENIC

REAGENTS

(a) *Molybdenum blue for arsenic.*—Follow the procedure under Method A for preparing the molybdenum blue phosphorus reagent but use 12 grams of molybdic oxide per 250 ml. of H_2SO_4.

(b) *Standard arsenate.*—Prepare aqueous monopotassium arsenate solutions equivalent to 1, 10, 100, etc., gamma of arsenic per ml.

Other reagents.—See Method A.

DETERMINATION

Have the aliquot free of nitrate and phosphorus and the arsenic in the pentavalent form. (A previous separation by trichloride distillation into water followed by evaporation of the distillate with an excess of HNO_3 to the complete elimination of HCl and HNO_3 is probably the most satisfactory (14).) Dissolve and wash the resulting residue with hot water into a 50 ml. flask, neutralize to quinaldine red as directed under Methods A and B, make to approximately 30 ml., add 0.5 ml. of the molybdenum blue arsenic reagent, digest on the steam bath 30 minutes to develop the color, cool, make to volume, mix, and read as directed in Method A.

APPLICATION OF THE IMPROVED REAGENTS

The following experiment illustrates the advantage of the improved reagents over the original Zinzadze reagent for determination of phosphorus in the presence of salts of appreciable buffer capacity. Standards were prepared containing phosphate alone and phosphate plus 0.38 gram of sodium acetate and 0.072 gram of acetic acid, the quantities present in 20 ml. of Fisher and Thomas's soil extractant (19). These were treated by the original Zinzadze procedure and the procedures outlined under Methods A and B. The transmission curves, established by photometer reading, for the respective color intensities are given in Figure 1. The values are practically identical for both pure standards and standards plus extractant from zero to 30 gamma in both Methods A and B, whereas the Zinzadze method discloses a "blank" ranging from 1.5 gamma at zero to nothing at 40 gamma.

The following data from blank determinations in the presence of varying quantities of sodium silicate demonstrate the extent of silica interference in the improved procedure:

SiO_2 in 50 cc. aliquot (mg.)	50	100	150	200	250
Phosphorus equivalent of color (gamma)	0	.5	.4	.7	.8

The results were obtained with the usual 30 minute period of color development. Even the smallest of the above quantities of silica, i.e., 50 mg., is materially in excess of the quantity likely to be encountered under most conditions. With the original Zinzadze reagent much greater interferences from silica were observed, as it gives colors readily discernible to the unaided eye. With either reagent much greater interference occurs if

the development of the color is extended beyond 30 minutes, 50 mg. of silica giving a color equivalent to that of 20 gamma of phosphorus at the end of an hour.

The reagents described here are subject to iron, arsenic, and nitrate interference when these substances are present in excess of 10 mg., 1 mg., and 1 mg., respectively. Zinzadze (33) reported much higher limits of tolerance, but they could not be verified by tests made in the present studies.
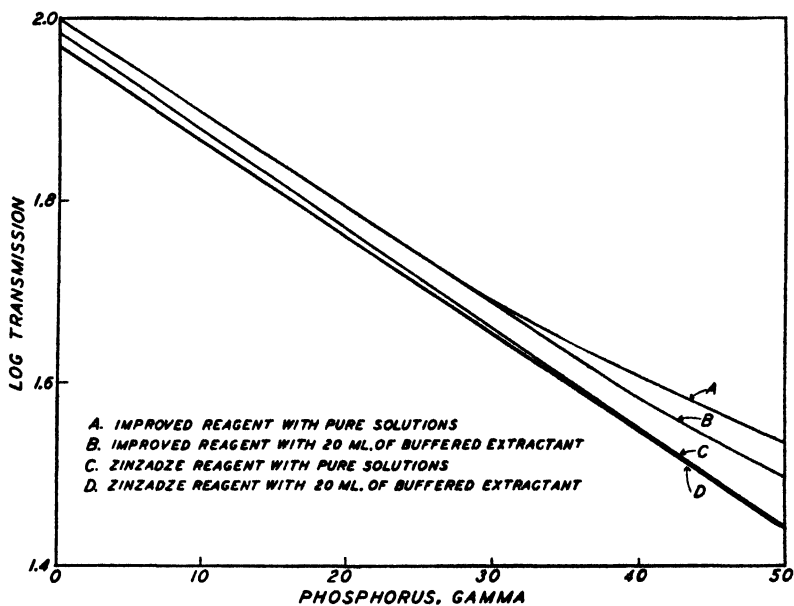
FIG. 1.—TRANSMISSION CURVES FOR COLOR INTENSITIES OBTAINED WITH THE IMPROVED AND THE ZINZADZE MOLYBDENUM BLUE REAGENTS IN PHOSPHATE SOLUTIONS WITH AND WITHOUT THE PRESENCE OF BUFFER SALTS

DISCUSSION

In the improved method Zinzadze's single reagent for both phosphorus and arsenic was replaced by separate reagents specifically adapted to combine optimum color development with minimum interference in the case of each. These reagents differ in molybdic oxide concentrations only. These concentrations (apart from that of Mo_2O_5) are 0.18 M and 0.32 M, respectively, for phosphorus and arsenic, as compared with Zinzadze's 0.25 M. The sulfuric acid concentration was increased from 25 N to 36 N (which also increased the H_2SO_4/MoO_3 ratio) in order to minimize the interference of silica and the buffer effect of salts. The reduction concentration (Mo_2O_5) was decreased from 0.1 N to 0.04 N to eliminate as much as possible of the yellow tints that prevent accurate reading of low concentrations. The substitution of quinaldine red for betadinitrophenol as an indicator for preliminary adjustment of the pH of the test solution further

insures that the final pH will remain well below 1.0 when the molybdenum blue reagent is added. Quinaldine red changes color from pH 3.5 to pH 2.0 and betadinitrophenol from pH 4.2 to pH 2.4 according to actual test upon buffered solutions with the glass electrode and the use of the photometer to register the range of color change. These figures are slightly at variance with those appearing in handbooks and other literature, but they illustrate the superiority of the former indicator in respect to sharpness of change as well as lower final pH. The "lemon yellow" betadinitrophenol end point advocated by Zinzadze occurs at about pH 3.0. Subsequent addition of the reagent does not bring the final pH below 1.0. For similar reasons the substitution of sulfurous acid for sodium bisulfite to eliminate interference from arsenic and nitrate is to be recommended, especially when the test solutions have a high salt or buffer concentration. Otherwise the final pH may exceed 1.0, the point at which blue color tends to develop in the absence of phosphorus or arsenic. As sulfurous acid is but slightly ionized it has little effect upon pH, whereas sodium bisulfite acts as a strongly alkaline buffer.

Since there is a considerable margin of safety with respect to the relative concentrations of the ingredients of the improved molybdenum blue reagent, the extreme care advised by Zinzadze in the preparation of the original reagent is unnecessary. It may be prepared in less than 30 minutes.

While the determination of phosphorus with the modified molybdenum blue reagent involves the development of color at a minimum of 95° C. for 30 minutes, and while this is more time-consuming than other procedures by which color is developed at room temperature in a few minutes, there are compensatory advantages in the attainment of a definite end point at a maximum of color density and stability. The rapidity of color development could be increased by lowering the acidity or increasing the molybdic oxide concentration, but at the expense of interference from silica, salts, etc. The time for development depends greatly upon the efficiency of the steam bath and should be established by the individual analyst. The figures given here apply to a steam bath that is capable of bringing 35 ml. of solution in a 50 ml. flask from room temperature to 95° C. in 10 minutes.

The concentrations of the ingredients of the sulfomolybdic acid reagent with metol as a reducing agent (Method B) are based upon those of the molybdenum blue phosphorus reagent. At the acid concentration used the metol acts specifically upon the phosphomolybdate formed. This reagent is excellent for phosphorus but unsatisfactory for arsenic. The sensitivities of the two phosphorus methods are practically identical, but Method B shows a somewhat more rapid development of color.

The 0.5 ml. of reagent should be measured with reasonable care by means of a graduated 1.0 ml. pipet. The reagent has less viscosity than

has that of Zinzadze and therefore may be pipetted directly without pre-liminary dilution. A deficiency of 0.1 ml. of reagent will result in a slight decrease in color, but an excess of 0.1 ml. is without significant effect. The quantity of reagent specified is capable of handling up to 80 gamma of phosphorus and nearly 200 gamma of arsenic, the color from which is far beyond readability. The limit of applicability of Beer's law is approxi-mately 30 and 75 gamma of phosphorus and arsenic, respectively, in 50 ml., and from the standpoint of readability it is undesirable to exceed these limits. As is to be expected, equivalent weights of phosphorus and arsenic give the same depth of color when development is complete.

Over the optimum range (0–30 gamma of phosphorus in 50 ml.) the sensitivity of the method is almost entirely dependent upon that of the apparatus used for measuring the color intensity. With the photoelectric colorimeter results are accurate within a few tenths of a gamma at the upper limit of 30 gamma, and quantities as low as 0.5 ± 0.1 gamma may be read. Although color intensities equivalent to 20 gamma of phosphorus are optimum for visual colorimetry, it is possible to estimate accurately much fainter colors (2–5 gamma) because of the elimination of trouble-some yellow tints.

SUMMARY

Difficulties encountered in applying Zinzadze's method to the deter-mination of minute quantities of phosphorus in soil extracts and similar solutions were traced to buffer effects in the presence of appreciable salt concentrations and to the composition of the molybdenum blue reagent itself.

The ranges of concentration of sulfuric acid, molybdic oxide, and reduc-tion over which color intensity remains practically constant were deter-mined experimentally. The conditions for minimum interference of yellow tints, silica, and salt effects require the minimum reduction and molybdic oxide concentrations, and maximum sulfuric acid concentration com-patible with uniform and complete color development.

Modified molybdenum blue reagents meeting these requirements are described. These reagents, used in the proportion of one part per 100 parts of test solution, have a sulfuric acid concentration of 36 N, reduction concentration of 0.040 N, and molybdic oxide concentration of 0.18 M and 0.32 M for phosphorus and arsenic, respectively. In composition they con-form to 0.02 M $Mo_{10}O_{29}$ and $Mo_{17}O_{50}$ for phosphorus and arsenic, respec-tively.

Preparation of the reagents themselves requires little time and care, but on the other hand, temperature and time are important factors in actual color development up to the point where a final stable end point is reached. Great precision may be obtained by measuring the colors in a photoelectric colorimeter, as little as one gamma or less equivalent of phosphorus being readable.

Substitution of quinaldine red for betadinitrophenol as an indicator for preliminary adjustment of the pH of the test solution is recommended.

Sulfurous acid is recommended instead of sodium bisulfite as a preventive of arsenic and nitrate interference in highly buffered and salt-containing solutions such as soil extracts.

An alternative method for phosphorus, specifying a sulfomolybdic acid reagent corresponding in concentration to the molybdenum blue reagent and metol as a reducing agent, is also described.

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THE CITRATE SOLUBILITY OF DOLOMITE OF VARYING PARTICLE SIZE*

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Dolomite is now extensively used as a basic material for the preparation of physiologically neutral fertilizer mixtures. Its use for all fertilizer purposes has increased from 68,000 tons in 1929 to 294,000 tons in 1936 (15). It is likely that reports for 1937 and 1938 will show a continuation of this increase. Although considerable work has been done on the reactions of dolomite in mixed fertilizers (2, 3, 4, 8, 10, 12, 14) and on its effect in the soil (11, 9) there still remain many unsolved problems connected with its use for fertilizer purposes. Among these are (a) the effect of extremely fine subdivision of dolomite on its reactivity, and (b) the choice of a method and solvent for the estimation, in the laboratory, of the availability of dolomitic magnesium to plants when used alone or incorporated in a mixed fertilizer. In this paper are presented the results of a study of the effect of particle size on the citrate solubility of four dolomites and of a preliminary study of four solvents for the estimation of available magnesium derived from dolomite.

I. EFFECT OF PARTICLE SIZE ON THE REACTIVITY OF DOLOMITE

The effect of particle size on the reactivity of dolomite has been studied by Siems and Batton (19). They investigated the rate of decomposition of dolomite in normal hydrochloric acid at 90° F. and reported that no significant differences in the rate of decomposition were observed when the dolomites were ground to fractions finer than that passing a 100-mesh sieve. They found that 64–99 per cent of the <100 mesh dolomites were dissolved in 10 minutes. It would seem that this treatment was too severe to show differences in the finer, and hence more reactive, fractions. Differences in the reactivity of these fractions would not be noticeable under such conditions since immediate and practically complete solution would occur in most cases.

Neutral ammonium citrate was chosen as a solvent because (a) it would dissolve some dolomite of the largest particle sizes and still not dissolve too much of the smallest sizes, and (b) the technic for its use is already well established from its use in the determination of available phosphorus in fertilizers (16).

Four dolomites, analyses of which are given, were used in this study. The dolomite was crushed in a jaw crusher to a size conveniently

Composition of dolomites

No.	CaO *per cent*	MgO *per cent*	Neutralizing Value (CaCO₃ Equiv.)
12	27.68	19.20	97.1
88	30.75	21.23	107.6
90A	29.90	19.34	101.4
95	24.88	17.03	86.7

handled in a friction mill. After each passage through the mill the material was screened, and the larger particles were reground. This process was continued until sufficient material of each of the mesh sizes 20–40, 40–60, 60–80, 80–100, and 100–200 had been accumulated. The initial screening was done by hand, but all fractions were finally run 5 minutes each on a Ro-Tap machine to assure uniformity of the screen separations. The material that had passed the 200-mesh sieve was then further classified* by means of a Roller analyzer (17, 18) into fractions the particles of which had average diameters of 0–5, 5–10, 10–20, 20–40, and 40–74† microns. In adusting the analyzer the density of all the dolomites was assumed to be 2.9 grams per cc.

Two one gram samples of each fraction were digested one hour at 65° C. in 100 ml. of neutral ammonium citrate with shaking every 5 minutes as prescribed for available P_2O_5 (16). Magnesium was then determined in the insoluble residue and in the untreated fraction by solution in aqua regia and double precipitation of the magnesium as magnesium ammonium phosphate, essentially as recommended by Hoffman (7). All analyses were made in duplicate except as noted. As a check on the procedure, total MgO was determined in the Bureau of Standards Standard Dolomite and good agreement with the value given by that Bureau was obtained. The citrate-soluble MgO was obtained by difference. This difference, in the case of the larger sized particles was often so small as to be only 2 to 3 times the probable precision of the MgO determinations and the values for citrate-soluble MgO are therefore not of the highest accuracy in the coarsest fractions. Table 1 shows the results obtained for the entire range of particle sizes from the 20–40 mesh fraction to the 0–5 micron fraction. In Figure 1 the citrate-soluble MgO, expressed as per cent of the total MgO, is plotted against the particle size for those fractions coarser than 200 mesh, while the values for the fractions finer than 200 mesh are similarly plotted in Figure 2. The analyses of the intermediate fractions, 80–100 mesh to 20–40 microns, inclusive, were repeated on dolomites Nos. 88, 90A, and 95, but no significant variation from the original data was obtained. The figures given for these fractions are the average of the three sets of determinations.

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* The classification was made by the American Instrument Co. of Silver Spring, Md.
† The openings in a Taylor Standard U. S. Series Equiv. screen No. 200 are 74 microns wide.

TABLE 1.—*Effect of particle size on the solubility of four dolomites in neutral ammonium citrate*

PARTICLE SIZE		MAGNESIUM OXIDE CONTENT											
		DOLOMITE NO. 12			DOLOMITE NO. 88			DOLOMITE NO. 90A			DOLOMITE NO. 95		
MESH	AVERAGE DIAMETER	TOTAL	CITRATE-INSOLUBLE	CITRATE-SOLUBLE	TOTAL	CITRATE-INSOLUBLE	CITRATE-SOLUBLE	TOTAL	CITRATE-INSOLUBLE	CITRATE-SOLUBLE	TOTAL	CITRATE-INSOLUBLE	CITRATE-SOLUBLE
	microns	per cent	per cent of total MgO	per cent of total MgO	per cent	per cent of total MgO	per cent of total MgO	per cent	per cent of total MgO	per cent of total MgO	per cent	per cent of total MgO	per cent of total MgO
20– 40	833–381	19.42	97.9	2.1	21.22	96.6	3.4	17.12	93.2	6.8	17.51	90.1	9.9
40– 60	381–246	19.32	95.6	4.4	21.07	96.2	3.8	16.90	90.3	9.7	17.26	90.3	9.7
60– 80	246–175	19.31	94.7	5.3	21.08	94.7	5.3	17.04	88.4	11.6	17.23	89.1	10.9
80–100	175–147	19.32	94.1	5.9	21.06	94.9	5.1	17.26	88.1	11.9	17.51	89.3	10.7
100–200	147– 74	19.46	92.6	7.4	21.23	93.5	6.5	17.37	85.7	14.3	17.64	87.1	12.9
	40– 74	20.19	91.9	8.1	21.34	94.9	5.1	17.77	87.2	12.8	17.50	91.9	8.1
	20– 40	20.14	88.7	11.3	21.08	92.5	7.5	17.68	81.3	18.7	17.13	88.3	11.7
	10– 20	20.19	84.6	15.4	20.44	89.7	10.3	16.44	78.3	21.7	18.05	84.0	16.0
	5– 10	19.52	77.7*	22.3	20.58	79.1	20.9	15.53	69.2	30.8	16.50	74.8	25.2
	0– 5	18.34	58.9*	41.1	20.44	58.9*	41.1	14.61	49.1	50.9	14.22	52.9	47.1

* Not run in duplicate owing to lack of material.

DISCUSSION

In every case the total magnesium became definitely less in the finest fractions. This may have been due to concentration of impurities in these fractions, which would have the effect of diluting the sample. This
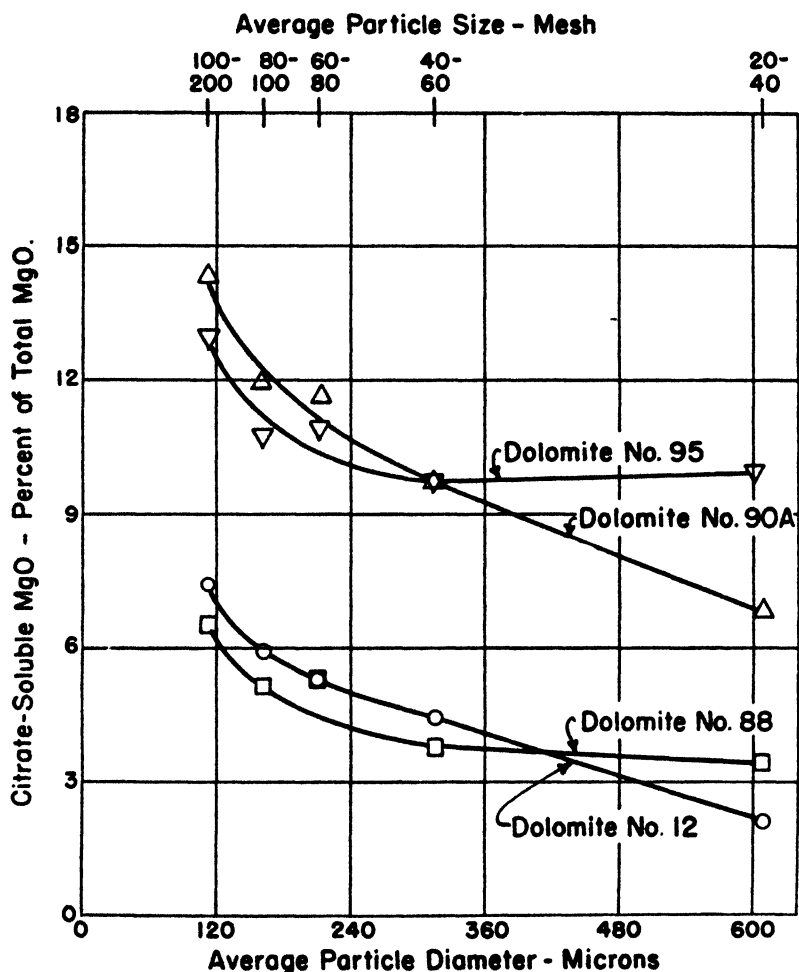
FIG. 1.—CITRATE SOLUBILITY OF MgO IN DOLOMITE FRACTIONS ABOVE 200 MESH

view is in part supported by the observation that the least pure of these dolomites, No. 95, showed the greatest decrease, 3.29 per cent, in total MgO in passing from the 20–40 mesh fraction to the 0–5 micron fraction.

The citrate-insoluble MgO shows a slight but definite trend downward in passing from 20–40 to 100–200 mesh in all four dolomites. This trend be-

comes very pronounced in the <200 mesh fractions, but the coarsest fraction obtained on the analyzer, 40–74 microns, in every case except No. 12 contained slightly more citrate-insoluble MgO than did the corre-
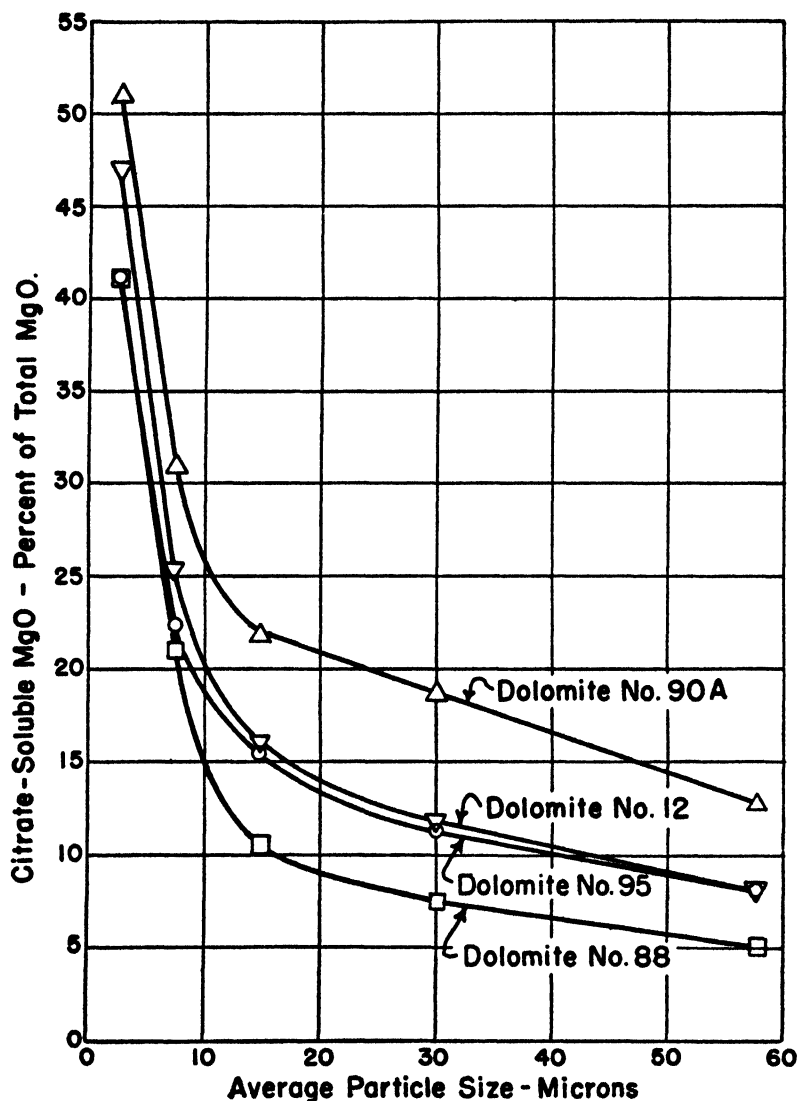
FIG. 2.—CITRATE SOLUBILITY OF MgO IN DOLOMITE FRACTIONS BELOW 200 MESH

sponding 100–200 mesh fraction. This, of course, is contrary to expectations and is probably best explained by the inherent differences in the two methods of classification, screening, and air flotation as used in the Roller

analyzer. Apparently the curve for the <200 mesh fractions is shifted upward slightly from where it would be if these fractions had been obtained by a technic governed by the same laws that are operative in obtaining screen fractions. A complete discussion of this subject is beyond the scope of this paper.

The significance of the results is perhaps best appreciated when they are expressed as per cent of the total MgO that was citrate soluble in the various fractions. Table 1 and Figures 1 and 2 show that this citrate solubility is approximately doubled in each case in passing from 20–40 to 100–200 mesh and that a further fivefold increase (approximately) occurs in passing from the 40–74 to the 0–5 micron fraction. Most of the latter increase occurs below 15 microns. The increased solubility obtained by decreasing the particle size from 20–40 mesh to 100–200 mesh is thus relatively small compared with the big increase obtained in the finer fractions. The apparent discrepancy between the 100–200 mesh and the 40–74 micron fractions mentioned above is, of course, also reflected in the citrate-soluble figures but has no important effect on the conclusions reached.

Whether or not the increased reactivity resulting from very fine subdivision is necessary or even desirable from the standpoint of availability to plants and its effect on the soil reaction remains to be determined. It is apparent, however, that the rate of the reactions that take place, or may take place, between dolomite and other constituents of fertilizer mixtures could be modified by a suitable choice of particle size.

It has been the practice with some workers to classify dolomites into hard, medium or soft grades, depending on the reactivity or solubility (under standard conditions) of dolomite that has been ground to pass some particular sieve, often either 100 or 200 mesh. It is apparent from the data presented here that such a system of classification might result in a hard dolomite being rated as medium or even as soft if it were ground in such a manner that the <100 or <200 mesh material contained a high proportion of the finest sizes. The reverse might also be true for a soft dolomite ground so as to contain a relatively low proportion of fines. Dolomite No. 88, if ground to pass 200 mesh in such a manner that 80 per cent of the particles were 20 microns or smaller, would appear softer than the relatively soft dolomite No. 95 in which 40 per cent of the particles were actually in that size range.

The particle size distribution in the <200 mesh fractions of the four dolomites used here is given in Table 2. Although the grinding procedure used was uniform in all cases considerable variation in the relative amounts of the various fractions occurred. Thus, No. 12 has twice as much material falling into the 0–5 micron fraction as has No. 88, but only about one-half as much in the 40–74 micron fraction. Since the solubility of the 0–5 micron fraction is, in the more favorable of the two cases, five

TABLE 2.—*Particle size distribution in the <200 mesh fraction of four dolomites as determined by the Roller analyzer*

SIZE RANGE	DOLOMITE NO.			
	12	88	90A	95
microns	*per cent*			
40–74	18.2	35.7	19.1	18.8
20–40	32.7	30.6	32.6	37.9
10–20	20.9	15.6	18.6	20.7
5–10	11.6	10.0	15.3	12.0
0– 5	16.6	8.0	14.2	10.7

times that of the 40–74 micron fraction, it is obvious that this variation in particle size distribution can have considerable influence on the reactivity of the <200 mesh fraction.

II. SOLUBILITY IN FOUR SOLVENTS OF THE MAGNESIUM IN DOLOMITE

Smith (20, 21, 22) and Smith and Deszyck (23) have determined the solubility of dolomite and other forms of magnesium in various solvents with a view to using this property as a measure of the availability of the magnesium to plants. As pointed out by Smith, the final choice of a solvent and technic for the determination of availability cannot be made until the present very meager knowledge of crop response to various forms of magnesium has been greatly extended. It would be convenient if the final choice of solvent and procedure could be one also suitable for P_2O_5. Experiments were therefore carried out in which the new solvent recently suggested by MacIntire, Shaw and Hardin (13) was compared with neutral ammonium citrate, ammonium citrate of pH 4 and 2 per cent citric acid.

Three mixtures were made up, each consisting of one-half of 100-mesh dolomite No. 95 and one-half of either pure monocalcium phosphate, superphosphate, or double superphosphate. Enough water was added to each mixture to raise its total moisture content to 7 per cent. The mixtures were then intimately mixed by rubbing with a spatula and by being forced through a 30-mesh sieve to disintegrate all lumps and allowed to stand overnight in loosely stoppered bottles at room temperature before the one gram samples were weighed for the determinations. One-half gram samples of the dolomite alone were run for purposes of comparison.

Six samples of each mixture and of the dolomite were washed with water, as in the water-soluble P_2O_5 determination (16), and then two of each set of six were digested for 30 minutes at 65° C. with 2 per cent citric acid, two with ammonium citrate of pH 4 (prepared according to Smith (22)), and two with ammonium citrate of pH 7. Two other samples of each material were digested in "citrated ammonium nitrate," as recom-

mended by MacIntire, Shaw and Hardin. The apparatus used was that recently described by Adams (1). Instead of analyzing the solution as recommended in the case of P_2O_5, MgO was determined in the residue after washing with water at 65°. The results are given in Table 3.

TABLE 3.—*Solubility in four solvents of the MgO in dolomite and in mixtures of dolomite and phosphates*

MATERIAL	SOLVENT	SOLUBLE MgO (PER CENT OF TOTAL MgO)
Ca(H₂PO₄)₂ · H₂O + Dolomite	2% Citric Acid	97.11
Ca(H₂PO₄)₂ · H₂O + Dolomite	Ammonium Citrate *p*H 4	78.01
Ca(H₂PO₄)₂ · H₂O + Dolomite	Ammonium Citrate *p*H 7	31.13
Ca(H₂PO₄)₂ · H₂O + Dolomite	Citrated NH₄NO₃	91.44
Superphosphate + Dolomite	2% Citric Acid	95.00
Superphosphate + Dolomite	Ammonium Citrate *p*H 4	66.97
Superphosphate + Dolomite	Ammonium Citrate *p*H 7	22.09
Superphosphate + Dolomite	Citrated NH₄NO₃	73.49
Double Superphosphate + Dolomite	2% Citric Acid	84.16
Double Superphosphate + Dolomite	Ammonium Citrate *p*H 4	76.30
Double Superphosphate + Dolomite	Ammonium Citrate *p*H 7	30.87
Double Superphosphate + Dolomite	Citrated NH₄NO₃	85.45
Dolomite Alone	2% Citric Acid	98.83
Dolomite Alone	Ammonium Citrate *p*H 4	83.66
Dolomite Alone	Ammonium Citrate *p*H 7	23.69
Dolomite Alone	Citrated NH₄NO₃	86.41

Two per cent citric acid dissolved 99 per cent of the MgO in the dolomite alone and 84–97 per cent in the mixtures. Ammonium citrate of *p*H 4 dissolved 84 per cent in the dolomite alone and 67–78 per cent in the mixtures, while that of *p*H 7 dissolved only 24 per cent of the MgO in the dolomite and 22–31 per cent in the mixtures. The special citrated ammonium nitrate suggested by MacIntire was slightly more reactive than ammonium citrate of *p*H 4. It dissolved 86 per cent of the magnesia in the dolomite and 73–91 per cent of the MgO in the mixtures. As stated above, the final choice of a reagent and a procedure for estimating available magnesia must await the results of vegetative tests. The present work shows, however, that the above reagents vary in solvent power and it may be that one of them will be found suitable for estimating available MgO. The preliminary data of Dawson, Snyder, Leighty and Reid (6) and of Collins and Speer (5) indicate, however, a much higher availability to plants as measured by decomposition in the soil than the solubility in neutral ammonium citrate shows.

TABLE 4.—*Variation in solubility of dolomitic MgO in neutral ammonium citrate with age and composition of mixture*

NO.	MIXTURE§	TOTAL MgO IN MIXTURE	DOLOMITE ALONE	CITRATE-SOLUBLE MgO IN DOLOMITE MIXTURE AFTER STORAGE FOR		
				1 DAY*	21 DAYS†	46 DAYS†
		per cent		per cent of total MgO		
1	6-8-6 containing Dolomite No. 88	5.36	10.55	6.01	16.64	14.83
2	do Dolomite No. 90	4.90	29.88	20.39	33.35	28.08
3	do Dolomite No. 95	4.73	20.61	20.00	23.63	28.18
4	Ammoniated Double Super. and Dolomite No. 88	10.34	10.22	9.55	13.61	11.64
5	do Dolomite No. 90	9.45	25.02	22.06	26.27	26.95
6	do Dolomite No. 95	8.50	18.67	16.81	22.76	26.93
7	Ammoniated Superphosphate and Dolomite No. 88‡	10.08	10.22	7.24	10.81	10.34
8	do Dolomite No. 90	9.17	25.02	19.69	22.62	17.93
9	do Dolomite No. 95	8.28	18.67	15.45	18.43	20.77
10	Superphosphate and Dolomite No. 88‡	10.00	10.22	7.84	4.19	0.00
11	do Dolomite No. 90	9.18	25.02	22.95	23.18	23.53
12	do Dolomite No. 95	8.41	18.67	17.00	16.48	18.15
13	Double Super. and Dolomite No. 88‡	10.82	10.22	40.93	28.02	27.12
14	do Dolomite No. 90	10.50	25.02	39.62	46.48	38.19
15	do Dolomite No. 95	9.59	18.67	32.29	42.60	38.11
16	Ca(H₂PO₄)₂·H₂O and Dolomite No. 95‡	9.28	18.67	2.79	40.39	45.41

* At room temperature.
† At 60° C.
‡ Equal amounts of components.
§ 7 % H₂O added to all mixtures.

Ammonium citrate of *p*H 7 has been used extensively in this laboratory in a study of the reactions of dolomite in mixed fertilizers. The results obtained in a storage experiment, some of which are given in Table 4, indicate that this reagent is unsuitable for control work, due to the variable results obtained with mixtures of different age and composition. It is felt that during the growing season the MgO is probably about equally

available to plants in all cases in spite of the fact that its solubility in the neutral ammonium citrate varied widely. In the 6–8–6 mixture No. 1 containing dolomite 88 the reagent indicated the MgO to be only 6.0 per cent soluble in the fresh mixture, whereas it became 16.6 per cent soluble after 21 days and was 14.8 per cent soluble after 46 days. The solubility of the MgO in this dolomite alone, when there was used a sample of a size corresponding to the amount of dolomite in a one gram sample of the mixture, was 10.6 per cent. Its solubility when included in various fertilizer mixtures ranged from 6 to over 40 per cent. Similar variations in solubility in this reagent have been noted by Smith and Deszyck (23) and by Beeson and Ross (4). No doubt real differences in the solubility existed and were measured with more or less accuracy by this reagent, but its extreme sensitivity to the age and composition of the mixture makes it unsuitable for use in the routine determination of the solubility of magnesium.

The other three reagents, 2 per cent citric acid, ammonium citrate of *p*H 4 and citrated ammonium nitrate, should give more uniform results, due to the fact that the solubilities of the MgO in these solutions are much higher than they are in ammonium citrate of *p*H 7 and alterations in the state of combination of the magnesia in the fertilizer would therefore have less effect.

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IMPROVED METHOD FOR ESTIMATING
CAROTENE IN FEEDS

By G. S. FRAPS and A. R. KEMMERER (Agricultural
Experiment Station, College Station, Texas)

In several recent papers Cary, Shinn, and associates (3, 4) have shown by chromatographic adsorptive methods that the carotene solution usually prepared in the estimation of carotene is not pure but may contain from 10 to 30 per cent coloring matter, not carotene. Their method consists in removing the impurities from the petroleum ether solution of carotene by specially activated magnesium hydroxide. The solution of carotene is passed through a column of magnesium hydroxide held in a condenser cooled by ice water and in an atmosphere of nitrogen. The method has not been published, but the authors kindly furnished information regarding it.

The attempts of the writers to use this chromatographic method were not successful, since the magnesium hydroxide prepared adsorbed and held the carotene so tenaciously that it could not be completely washed out of the column by the petroleum ether (Skellysolve F, 86–170° F.). Therefore a study was made of the effect of different periods of heating, temperature, and water treatment upon the adsorptive power of magnesium hydroxide. The preparations obtained were tested for adsorption of carotene by a determination of the loss of color after 0.5 gram of the material had been shaken with 10 cc. of petroleum ether containing about 2 p.p.m. of purified carotene and also for adsorption of xanthophyll (methanol fraction of carotenoid pigments) by use of a similar solution of crude xanthophyll prepared from alfalfa leaf meal.

Some of the treatments and the results are given in Table 1. These data show that two of the preparations did not adsorb carotene but retained all the xanthophyll, while the other preparations adsorbed from 5 to 95 per cent of the carotene. These results were confirmed by additional work in which there were used 2.5 grams of adsorbent and 50 cc. of carotene solution and the color was measured by means of the KWSZ photometer, a much more accurate test than is visual comparison with bichromate. Portions of a preparation of magnesium hydroxide that did not adsorb carotene but did adsorb xanthophyll were shaken with carotene preparations from various samples, with the results given in Table 2. Since this preparation does not adsorb carotene, the color removed is evidently due to impurities. The carotene estimated in the original solution is hereafter termed "crude carotene" and that in the solution shaken with magnesium hydroxide is termed "pure carotene." The percentage of impurities in the crude carotene ranges from 0 to 23 per cent.

The use of properly prepared magnesium hydroxide offers a simple method for purifying carotene solutions and increasing the accuracy of

TABLE 1.—*Effect of water and heat treatment on the adsorptive power of U.S.P. light MgO*

LABORATORY NUMBER	WATER ADDED TO 50 GRAMS OF MgO	HEATED ON STEAM BATH	HEATED IN ANILINE VAPOR	CAROTENE ADSORBED	XANTHOPHYLL ADSORBED
	cc.	minutes	minutes	per cent	per cent
50207	50	30	120	95	100
50209	50	120	30	90	100
50208	25	120	30	85	100
50211	50	120	120	85	100
50210	25	120	120	80	100
50206	25	30	120	80	100
50379	50	120	0	75	100
50204	25	30	30	70	100
50205	50	30	30	55	100
50381	75	30	30	5	100
50378	50	30	0	0	100
50380	75	30	0	0	100
50377				40	70

TABLE 2.—*Crude and pure carotene in alfalfa products*

LABORATORY NUMBER	SAMPLE	CAROTENE BY BICHROMATE			CAROTENE BY KWSZ PHOTOMETER		
		CRUDE	PURE	PER CENT PURITY	CRUDE	PURE	PER CENT PURITY
		p.p.m.			p.p.m.		
49850	Old alfalfa hay	25.0	20.7	82.6			
49851	New alfalfa hay	24.3	20.2	83.1			
50252	Dehydrated alfalfa leaf meal	150.0	145.5	97.0			
50529	Old alfalfa leaf meal	9.0	7.1	88.2	10.2	8.4	82.4
48481	Dehydrated greens	170.0	137.5	80.9	172.0	148.0	86.0
50541	Dehydrated alfalfa leaf meal	75.6	60.0	79.4	76.1	64.3	84.5
50337	Dehydrated alfalfa leaf meal	90.0	90.0	100.0			
50346	Alfalfa meal	16.6	13.4	80.7			
50743	Alfalfa leaf meal	280.7	275.7	98.2	304.0	296.7	97.6
50783	Alfalfa leaf meal	110.8	100.8	91.0	115.0	112.5	97.8
50784	Alfalfa leaf meal	55.0	45.4	82.5	58.4	47.4	81.2
50842	Dehydrated alfalfa leaf meal	96.7	74.4	76.9	99.5	78.3	78.7
50425	A.O.A.C. Sample 1 for carotene	240.0	207.1	86.3	237.5	205.0	86.3
50426	A.O.A.C. Sample 2 for carotene	78.9	75.6	95.8	77.3	73.3	94.8

the estimation of carotene. All that is necessary is to shake the crude carotene solution in petroleum ether with suitable magnesium hydroxide, separate the liquid, and read the color. Whether or not this carotene is

now entirely pure must be ascertained by further study, but it is certainly more nearly pure than it was before the treatment. This is called the method of selective adsorption. As stated previously, it depends upon the use of magnesium hydroxide that has been tested and found not to adsorb any carotene but to adsorb xanthophyll completely.

In order to test whether the carotene adsorbed by magnesium hydroxide preparations that adsorb small amounts of carotene might be washed out and the method be modified so as to use such preparations, one gram portions of the magnesium hydroxides having various adsorptive powers for carotene were placed on cotton in a glass tube 8 mm. in diameter, 25 cc. of the petroleum ether containing about 4 p.p.m. of pure carotene was passed through it with the aid of gentle suction, and the contents of the tube were then washed with 65 cc. of the solvent. The results are shown in Table 3. As the adsorbed carotene was not washed out under these conditions, it is evident that magnesium hydroxide that adsorbs even small quantities of carotene cannot be used for purifying carotene. Even when the tube was surrounded with ice water and the

TABLE 3.—*Adsorption and retention of carotene by a column of the adsorbent of different grades*

LABORATORY NUMBER	GRADE AS SHOWN BY CAROTENE ADSORBED IN SHAKING WITH ADSORBENT PERCENTAGE ADSORBED	ADSORPTION AND RETENTION IN A COLUMN OF THE ADSORBENT AFTER PERCOLATION AND WASHING PERCENTAGE ADSORBED
50790	30.4	28.7
50791	79.7	90.8
50792	70.6	57.3
50794	82.2	71.4
51075	7.1	12.2

experiment was carried out in an atmosphere of nitrogen, as was done by Cary, Shinn, et al. [1,2] the carotene could not be completely washed out. However, more carotene was recovered under such conditions than at room temperature and in air. The adsorbed carotene could easily be washed out completely by a mixture of ethanol and petroleum ether, but this solvent might wash out the impurities also.

In order to determine whether preparations of magnesium hydroxide of desired selectivity could be easily made, all available samples of magnesium oxide were tested (Table 4). Only one sample of the desired characteristics was produced, and this was merely a fortunate chance. The preparation of magnesium oxide is now being investigated and it should soon be possible to specify conditions that will assure a reliable source of supply. Some of the materials used to dilute adsorbents may themselves have considerable adsorptive power for carotene. According

[1] *Proc. Am. Chem. Soc., 95th Meeting, 1938*, B. p. 14.
[2] *Proc. Am. Soc. Biol. Chem.*, 31st Annual Meeting, 1937, 89.

to tests made by the writers, silica will adsorb 9.5 per cent of carotene from solution, siliceous earth 99.5 per cent, and infusorial earth 59.5 per cent.

TABLE 4.—*Effect of water and heat treatment on the adsorptive power of different kinds of MgO*

LABORATORY NUMBER	KIND OF MAGNESIUM OXIDE	WATER ADDED TO 50 GRAMS	HEATED ON STEAM BATH	HEATED IN ANILINE VAPOR	CAROTENE ADSORBED	XANTHOPHYLL ADSORBED
		cc.	*minutes*	*minutes*	*per cent*	*per cent*
50796	No. 1 U.S.P. grade	—	—	—	66.0	100.0
50790	No. 1 U.S.P. grade	50	30	—	30.4	94.2
50794	No. 1 U.S.P. grade	50	30	120	82.2	99.5
50797	No. 2 U.S.P. grade light	—	—	—	79.6	100.0
50791	No. 2 U.S.P. grade light	50	30	—	79.7	99.5
50793	No. 2 U.S.P. grade light	50	30	120	88.8	99.0
50798	No. 3 grade	—	—	—	100.0	100.0
50792	No. 3 grade	50	30	—	70.6	99.5
50795	No. 3 grade	50	30	120	92.4	100.0
50377	No. 4 U.S.P. grade light	—	—	—	40.0	100.0
50378	No. 4 U.S.P. grade light	50	30	—	0	100 0
50207	No. 4 U.S.P. grade light	50	30	120	95.0	100.0

The method of selective adsorption has heretofore been applied chiefly to alfalfa products. When it is applied to other materials an adsorbent having different selective powers or a modified procedure may be required. For example, a different procedure is required for the determination of carotene in rat excrement. The writers have shown[1] that the excrement of rats and of chickens fed on a diet free from carotene contains other coloring materials that may interfere with the determination of carotene. It was also found that the coloring matter in the rat excrement is not adsorbed even by such magnesium hydroxide that adsorbs practically all the carotene in solution. Therefore, for rat excrement, it may be possible first to purify the carotene solution by selective adsorption and read the color. Then all the carotene can be removed by a second treatment, the color again read, and the last value subtracted from the first value. The remainder will be the value for pure carotene. The application of the method to various foods, feeds, and other materials containing carotene is now being studied, as is also the possibility of the applicability of the principle of selective adsorption to a large number of coloring materials and to the purification of carotene and other compounds.

The method follows:

[1] *J. Nutrition*, 16, 309–15 (1938).

METHOD OF SELECTIVE ADSORPTION
REAGENTS

(a) *Magnesium hydroxide.*—Place 50 grams of suitable (U.S.P. light) MgO in an evaporating dish, add 50 cc. of water, and heat on a water bath for 30 minutes. Shake 0.5 gram of this activated material with 10 cc. of purified carotene solution, or 2.5 grams with 50 cc., and allow the Mg(OH)$_2$ to settle, or centrifuge. Read the color by the usual method both before and after the treatment. Test in the same way with the xanthophyll solution. If the Mg(OH)$_2$ adsorbs all the xanthophyll and none of the carotene, it is suitable for use. If it does not, try another lot of MgO.

(b) *Carotene solution.*—Dissolve 0.1 gram of pure carotene in 2 cc. of CHCl$_3$ and precipitate with about 25 cc. of methanol. Filter off the carotene and dry between filter paper and in a vacuum desiccator. Weigh out 20 mg. of the carotene, dissolve in a few drops of CHCl$_3$, and make up to 50 cc. with light petroleum ether. Dilute 5 cc. of this solution to 1000 cc. with light petroleum ether.

(c) *Xanthophyll solution.*—Saponify about 6 grams of alfalfa with 120 cc. of alcoholic potash and extract with U.S.P. ethyl ether as directed in the published method for carotene.[1] After the ethyl ether is evaporated off, take up the residue in light petroleum ether and wash with 90% methanol until the methanol comes through colorless. Then extract the methanol fraction two times with petroleum ether to remove traces of carotene. Extract this petroleum ether fraction with 90% methanol as directed above and add the methanol extract to the original methanol solution. Evaporate off the methanol with diminished pressure and take up the residue in 100 cc. of petroleum ether. Dry over anhydrous Na$_2$SO$_4$ and dilute to contain the equivalent of 2 p.p.m. of carotene.

DETERMINATION

Prepare the carotene solution in the usual way, read the color, and estimate crude carotene if desired. If necessary, adjust the solution to contain about 2 p.p.m. of the color equivalent to carotene. Shake the solution with the Mg(OH)$_2$ at the rate of 0.5 gram to 10 cc. and read the color by the usual method. Report as pure carotene in p.p.m. (The Mg(OH)$_2$ used must have the correct selectivity, that is, it must, when tested, adsorb xanthophyll from solution completely and adsorb no carotene.)

Note on the Rapid Determination of Mineral Oil in Butter[*]

In the determination by the F.A.C. method[2] of unsaponifiable matter in butter that was suspected of containing mineral oil, it was noted that a milky emulsion formed on the addition of water, after saponification, in those samples that contained mineral oil, whereas a perfectly clear solution was obtained in the case of pure butterfat. This fact was made the basis of a rapid test for mineral oil in butter. This test is not new. It is given in essentially the same form in the book of D. Holde, titled, "The Examination of Hydrocarbon Oils and of Saponifiable Fats and Waxes," translated by Edward Mueller, second English edition, p. 76. Substantially the same test is given in *Methods of Analysis, A.O.A.C.*, 1935, p. 89, 9.

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[1] G. S. Fraps and A. R. Kemmerer, Texas Agr. Exp. Sta. Bull. 557, pp. 1–28 (1937).
[*] By J. H. Bornmann, U. S. Food and Drug Administration, Chicago, Ill. Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., November 14, 15, and 16, 1938.
[2] *Methods of Analysis, A.O.A.C.*, 1935, 420, 35.

The writer made the test on a 2 cc. sample of fat, using the same proportion of alcohol and potassium hydroxide (1+1) as that given in the F.A.C. method, but heating only 10 minutes. It was found that amounts of mineral oil smaller than 2 per cent could not be detected. It was then discovered by E. H. Wells that when the proportion of alcohol was increased it was possible to detect as little as 0.5 per cent of mineral oil. The test follows:

Place about 10 grams of butter on a folded filter, heat in an oven until the butter is melted, and filter in a warm place. Measure 1 cc. of clear fat, 1 cc. of KOH (1+1), and 25 cc. of alcohol (95%) into a test tube (8″×10″). Heat the tube and contents in a water bath at 80° C. for 15 minutes, with sufficient shaking to mix the contents. Dilute with an equal volume of distilled water.

A turbidity after dilution with distilled water indicated the presence of mineral oil. A pure butterfat run simultaneously as a control remains perfectly clear on dilution. It is necessary, of course, to verify the presence of mineral oil, in case of a positive test, by the usual determinations of constants.

BOOK REVIEWS

Statistical Methods. By GEORGE W. SNEDECOR, Director, Statistical Laboratory of Iowa State College and Head of the Statistical Section of the Iowa Agricultural Experiment Station. Collegiate Press, Inc., Ames, Iowa. 1937, revised 1938. 388 pp. Price $3.75.

In most of the newer textbooks on statistical methods, insufficient attention has been given to the treatment of small samples of experimental data. Mr. Snedecor's book stresses the methods to be used in the analysis of small samples in experiments in the natural sciences. Special emphasis has been placed upon the application of the chi-square test, tests of significance, measurement of degrees of freedom, analysis of variance and co-variance and correlation.

Mr. Snedecor has worked closely with Dr. R. A. Fisher of the Rothamsted Experiment Station in England, and his book closely parallels the book of Dr. Fisher on "Statistical Methods for Research Workers." Mr. Snedecor's book is much easier reading and follows Fisher's method of numbering paragraphs.

One of the more desirable characteristics of the book is the emphasis placed on the necessity of measuring the error of results based on samples. These tests of error are woven into all of the various parts of the text, and their proper application to the various statistical problems involving sample data is illustrated by many practical problems. Each step is also accompanied by examples for use in class work which point out the application of the different methods to various types of problems.

The necessity for applying both variance and correlation analysis to specific problems and the manner in which these two methods of analysis supplement each other have been given special consideration, which is a valuable contribution to the existing methods of statistical analysis. Another desirable feature of the book is that unusual applications of the various methods to experimental data have been segregated and a short course in the elements of Statistical Methods is outlined at the beginning of the book. This enables the reader to concentrate on the important sections without reading the whole book.

One of the chief criticisms of the book from the standpoint of a complete statistical text is that too little attention has been given to the methods of treating a large number of observations and to the presentation of statistical results. The book contains only one short chapter dealing with large sample theory. No attention at all is given to the proper methods of table construction. Only three paragraphs are devoted to graphic presentation and these are more on the presentation of results than the method of construction. Frequency distribution, averages, and dispersion are too briefly discussed to familiarize the student with the problems involved and the short cuts available in their computation. Correlation is more thoroughly treated, and the discussion is carried through multiple correlation and the presentation of curvilinear regressions. However, the treatment of curvilinear correlation is elementary and confined to the use of logarithms and polynomial curves.

Another weakness of the book as a reference or textbook is the use of symbols which, in many cases, are entirely different from other statistical textbooks and journals. For example, in correlation, E is used to represent the estimated value of the dependent variable and the formulas for other statistical measures are stated in terms of the betas.

References are incomplete and are confined to a great extent to publications by the Iowa Agricultural Experiment Station and by R. A. Fisher. While the text is written in a very simple style, many readers feel it is too simple and seems to underestimate the intelligence of the reader.—C. M. PURVES.

Cattle Fodder and Human Nutrition. By ARTTURI I. VIRTANEN. 108 pp. The University Press, Cambridge, England. The Macmillan Company, 60 Fifth Ave., New York, N. Y., 1938. Price $2.25.

This book is the outgrowth of a series of four lectures. The first was delivered at the Universities of London and Cambridge, the second and third were given at the University of London, while the fourth was presented at the University of Reading.

Lecture I. The Mechanism of Biological Nitrogen-Fixation. By a description of methods used and results obtained, the author has led to a logical discussion of a possible chemical mechanism of nitrogen-fixation by free-living and symbiotic nitrogen-fixing organisms.

Lecture II. The Symbiosis of the Leguminous Plants and Legume Bacteria. In the second lecture, with less emphasis on the mechanics of the process, Professor Virtanen has described experiments designed to show the interdependence of the symbiotic organisms and host plants. Several tables give data in support of his discussion of the subject.

Lecture III. The Production of Vitamins in Agriculture with Special Reference to Human Nutrition. This chapter is essentially a report of a study made under the direction of the author on the standard of nutrition of working classes in Finland. Considerable space is devoted to the Vitamin-A potency of milk and milk products and the need of increasing it by methods of cattle feeding.

Lecture IV. The A. I. V. Method for the Preservation of Fresh Fodder and Its Importance in Agriculture. Perhaps the best of the four lectures, this is a thorough discussion of both the theoretical and practical aspects of the production and use of A. I. V. fodder.

The title of this book may appear, at first, to be misleading. Further consideration and reading of the complete text, however, will show that the author has developed logically the subject of nitrogen-fixation, which, of course, has a direct bearing on the food value of forage crops. This naturally leads to the problem of the preservation of fresh fodder, and finally, to the part it may play in the production of vitamins, indirectly, for human consumption. The fact that the last two subjects are discussed in the reverse order does not in the least detract from the value of the book.

The book is written in a clear, straight-forward style and should be of interest to students of general agriculture. One hundred and four references, many of them to research published by the author, will assist students in specialized fields who may desire to pursue the subjects further.—G. CHAPMAN CROOKS.

Micropedology. By WALTER L. KUBIENA. 237 pp. Illustrated. Collegiate Press, Inc., Ames, Iowa. Price $3.00.

Kubiena presents his well-known work on the microscopic study of soils in this book, which has been produced in English as a result of his lecture courses at Iowa State College. The attitude of the advocate, or even that of the propagandist, is often evident. Out of 237 pages, 35 cover introduction and description of apparatus; 40 are devoted to micromanipulation and sampling, about 40 to a selection of more or less standard microchemical formulas, optical methods, and reactions, about 75 pages to soil "fabric," and 30 pages to the micropopulation of the soil.

There are two main points in his teaching: (1) That the microscope must go to the field if possible, but if not taken to the field, must be used to examine selected and representative soil masses in undisturbed condition fresh from the field or after subjection to known conditions; (2) that in doing this, microscopes having "incident" instead of transmitted light are needed and should be mastered by the student. Both ideas are desirable in any microscopic program as related to the soil.

His own soil microscope is fully illustrated along with other apparatus which may be substituted. The preparation and use of microtools is detailed.

The last 100 pages are devoted to the application of his methods to soil samples. Here his discussion of types of soil "fabric" is given greatest attention. By "soil fabric" he appears to mean what may perhaps be called the pattern formed by the arrangement of the granular, principally mineral components of the soil as modified by more or less complete envelopment in colloidal materials. A series of type names is given to these fabric types with the manipulations and tests necessary to identify and describe them. Many illustrations are presented to reenforce the descriptive text. The discussion of these types goes far enough to open up this largely neglected field, which will need many workers and a considerable period of time before others can safely take up Kubiena's boast that he can tell from the examination of a vial of soil properly taken, the climate, the general soil type, and many details of the area sampled.

Applied to the microorganisms of the soil, the method aids in a general understanding of space relations and the clumping and colonization of microorganisms. Those of us who have travelled with the author and have seen his demonstration, realize that the field microscope is only an interesting accessory to microbiology. It can not replace the usual types of culture.

Those familiar with microscopy as applied to many fields will appreciate this book as an attempt to adapt this form of examination to the study of soil. There is reason to expect that the development of soil microscopy will furnish supplementary information of considerable value to those describing soil types.—CHARLES THOM.

Utilization of Fats. By H. K. DEAN, Ph.D. (Liv.) A.I.C.; New York, 1938; Chemical Publishing Co. of N. Y., Inc. Pp. 292+index. Price $6.00.

In this book the author describes first the constituents of fats, methods for their analysis, their classification, and composition.—He then takes up the preparation of artificial glycerides and the hydrogenation, extraction, refining, biochemistry, and rancidity of fats. In the final portion, paint and varnish oils, soaps and pharmaceuticals, and cosmetic, lubricant, leather, and sulfonated fats are discussed.

The reviewer feels that the title of this book may be found somewhat misleading, as it is obviously impossible to give full treatment to the subject "Utilization of Fats" in 292 pages. Actually only slightly over half of the pages is given to this subject. The book gives an excellent summary of the developments in fat chemistry and technology during the last decade. It accomplishes the primary consideration of the monograph, which is to present a clearer picture of the relation of the structure of fats to their utilization. A satisfactory list of references follows each part of each chapter.

This survey will be a valuable aid to workers in the field of fat and oil chemistry. It should be used, together with the standard works listed by the author dealing with these fields, which, although lacking in the newest developments, yet do contain a wealth of useful detail which is necessary in an investigation of the utilization of the various fats.—R. S. McKINNEY.

John Phillips Street, 1869–1938

JOHN PHILLIPS STREET

John Phillips Street was born January 30, 1869, in Beverly, N. J. He graduated from Rutgers College as a Bachelor of Science in chemistry and earned the Phi Beta Kappa key.

He entered the service of the New Jersey Agricultural Experiment Station at New Brunswick. In 1907, he went to the Connecticut Agricultural Experiment Station at New Haven, taking the place of A. L. Winton, who had entered the service of the Bureau of Chemistry of the United States Department of Agriculture.

During the war Street received a commission in the army in connection with food work. While in France he was reported as having been killed in action but this report was subsequently found to be erroneous, the unfortunate person being another John Street. Street retired from the army with the rank of major and went back to Connecticut Shortly after this he resigned and went to Indianapolis to take charge of inspection work in that state for the National Canners Association, holding this post for three years He then moved to Rochester as Executive Secretary of the New York State Canners Association.

He died in Rochester on September 22, 1938 He is survived by his mother, his wife, son, two grandchildren, his sister, and two brothers.

Street was an extremely valuable member of the Association of Official Agricultural Chemists, but did not attend the meetings for about the last twenty years of his life, during which period he was not doing official agricultural work, and for this reason was unknown to many of the younger members of the present generation. He attended his first meeting of the Association in 1890 (7th annual meeting), and with only one or two exceptions attended every subsequent meeting through the 34th meeting in 1917. His first work for the Association was a comparison of results on total phosphoric acid by magnesia mixtures of different composition (U. S. Dept Agr., Div. Chem. Bull. 47, p. 62), presented at the 12th annual meeting in 1895.

At the 1896 meeting Street presented a Report on Nitrogen (U. S Dept. Agr., Div of Chem. Bull 49, pp 12-24), in which he first described the well-known Ulsch-Street method for determining nitric nitrogen At the 1897 meeting as Reporter on Nitrogen Street presented his second Report on Nitrogen (U. S Dept. Agr., Div. Chem. Bull. 51, pp 15 26), and the Ulsch-Street method was made official (p. 29).

Street was appointed a member of the Abstract Committee for 1898, 1899, 1900, and 1901. He was elected Vice President for the 1906 meeting and President for the 1907 meeting. He was Chairman of Committee A on Recommendations of Referees at the 1905 meeting. He presented a paper on "The Detection of Peat in Commercial Fertilizers" (U. S. Dept. Agr., Bur. Chem. Bull. 105, pp. 83-85). In 1907 he was also Associate Referee on Cattle Feeds.

Street's Presidential Address for the 1907 meeting at the Jamestown Exposition, Norfolk, Va., is published in Bur. Chem. Bull. 116, pp. 28 34. In 1908 he presented a report as Referee on the Determination of Acidity in Cattle Feeds (Bur. Chem. Bull. 122, pp. 160-163).

He was Associate Referee on Vegetables and a member of Subcommittee A on Recommendations of Referees for the year 1909-1910. At the 1910 meeting he presented his report as associate referee on this subject (Bur. Chem. Bull 137, pp. 122-134) and in association with C. B. Morison read a paper on "Ginger Extract" (*Ibid.*, pp. 76–79). He was appointed Chairman of the Appropriations Committee for 1911 and 1912, and Chairman of Subcommittee A on Recommendations of Referees for the year 1910–1911. He made reports as Chairman of this Subcommittee for the years 1912, 1913, and 1914. At the 1912 meeting he was also Chairman of the Committee on Participation in the Eighth International Congress of Applied Chemistry. He continued as Associate Referee on Vegetables during 1911 and 1912.

He was a member of the Special Committee on Editing Methods of Analysis

for the years 1913, 1914, 1915, and 1916, and a member of the first Board of Editors of *The Journal* of the Association for Vols. 1 and 2 (1915–1917). He was also a member of the Committee on Cooperation with Other Committees on Food Definitions for 1914, 1915, and 1916.

At the time Street went to Connecticut the food reports of the Connecticut Agricultural Experiment Station were among the few which food chemists desired to obtain and retain for their files. The reputation of these reports after Street came to Connecticut suffered no diminution. He began a systematic study of materials on the market, proprietary and otherwise, intended to be used by persons suffering from diabetes, and the published analyses attained an international reputation. This was before the days of insulin and long before the arrival of the present dietetic methods of treating persons afflicted with this disease. While at New Haven he also conceived the idea of publishing a book upon these proprietary and secret remedies, the analyses of which were scattered in various reports of persons engaged officially in examining such products. This compilation was published by the American Medical Association and was of inestimable value to those who were desirous of obtaining accurate knowledge of certain specific articles of this character.

No one person is competent to write concerning the character of another person. His family, his business subordinates, his business superiors, his neighbors, his social friends, and his intellectual friends all see a different side of his character.

My first meeting with Street was at the 1906 meeting of the official chemists at which time I was introduced to him by Winton. So indelibly was his appearance impressed upon my mind that I can now see him as he sat on the platform as vice president during the presidential address of Hopkins.

We subsequently became well acquainted. We visited each other's laboratories, but not each other's homes. We roomed together at many meetings of the A.O.A.C. and also met frequently at the meetings of the New England Food Control Officials of which for many years Street was the Secretary. We met at a few meetings of the National Association of Food and Drug Commissioners and of the American Chemical Society.

At the New Orleans meeting of the American Public Health Association he told many humorous stories of his experiences in France during the war and endeared himself to many of my New England friends engaged in public health work.

After I had attended a few meetings of the Association of Official Agricultural Chemists Street made me a member of the "Old Guard," which name he gave to a group of members of the Association, of which there are but few left. The "Old Guard" met on one evening of each Association meeting and reminisced. This group included Wiley, Frear, Trowbridge, Patrick, Mitchell, Hortvet, Doolittle, Ladd, Ross, Brackett, Bigelow, and others who have passed on. An evening with this group was an event to be remembered. A recent letter received from Mrs. Street states that she often heard Jack speak of the "Old Guard."

Street had many fine personal characteristics. Perhaps that for which I most admired him was that he was no gossip. I never heard him make a disagreeable statement about a man behind his back; if he had anything to say about a man, he said it to his face. He had a keen sense of humor. After returning from the war he remarked to a friend that he certainly enjoyed the obituaries resulting from the erroneous report that he had been killed. I heard him testify in New York City in the so-called Collier case. In cross examination he was asked, "Where is the mind?" He replied, "Ask a phrenologist."

It was men like Street that were largely responsible for making the Association of Official Agricultural Chemists what it is today.

Old friend, you have passed away. I well remember your voice, your aquiline countenance, your black mustache, your black hair with the one gray hair of which you were so justly proud.   HERMANN C. LYTHGOE

REPORT ON ALCOHOLIC BEVERAGES

By J. W. SALE (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The analysis of alcoholic beverages was studied extensively during the past year, reports being submitted on fourteen topics. The general subjects given attention were malt, malt extract, malt adjuncts; beer; wines; distilled spirits, including whiskey and brandy; and cordials and liqueurs.

Methods for the analysis of beer and for the determination of carbon dioxide, volatile acids, sulfur dioxide, aldehydes, methanol, benzaldehyde, volatile esters, and the synthetic flavor, gamma undecalactone, in beer, wines, or distilled liquors were subjected to collaborative study. Methods for carbon dioxide and aldehydes were recommended for adoption as tentative methods. Study of the saponification of esters with lead acetate was discontinued, as it was found that lead acetate is ineffective in saponifying esters in acid form as they exist in wines. All the other subjects mentioned were recommended for further study next year, although in many instances the results obtained, when collaborative work was conducted, were gratifying. In one case, sulfur dioxide in alcoholic and carbonated beverages, it was found that the preparation of the samples for collaborative analyses presents a special problem, as the content of sulfur dioxide was found to change between the time of preparation and analysis of the samples.

The specific recommendations made by the Associate Referees follow.

RECOMMENDATIONS[1]

It is recommended—

(1) That the tentative pressure air method for the determination of CO_2 in beer (see p. 208) be adopted as tentative and further studied collaboratively with the object of making it official.

(2) That the Associate Referee on Beer study collaboratively the following tentative methods relating to beer (Chap. XIV):[2] (a) Extract in original wort; (b) real degree of fermentation; (c) total acid; (d) dextrin; (e) direct polarization; (f) pasteurization; and (g) chlorides; and that he also include hydrogen-ion concentration in this study.

(3) That methods for the determination of heavy metals (Fe, Cu, Pb), As, and fluorine be studied.

(4) That the viscometric method for the determination of the proteolytic activity of malt outlined by the associate referee last year (*This Journal*, 21, 160) and the edestin titration method (*Wochschr. Brau.*, 53, 297) be further studied.

(5) That the vacuum method for the determination of moisture in

flour (p. 206, 2)[2] be studied as to its applicability to the determination of moisture in malt adjuncts (p. 161, 53).[2]

(6) That a special study be made of methods for the determination of fat that will be applicable to corn grits and brewers' rice and flakes.

(7) That a study of the method for determining the extract in malt adjuncts be made and that consideration be given to the suggestions to use a portion of the malt in the boiling operations.

(8) That special study be made of the diastatic activity of malt.

(9) That the study of methods for the detection of adulteration of distilled spirits be continued.

(10) That the collaborative study of sulfur dioxide in beer and ale be continued and that the study be extended to include a collaborative study of sulfur dioxide in wines.

(11) That further collaborative work be done on the tentative method for the determination of benzaldehyde (p. 183, 55), volatile esters (p. 181, 46) and gamma-undecalactone (p. 181, 47) in cordials.

(12) That the study of saponification of esters with lead acetate be dropped.

(13) That the distillation procedures for volatile acids in wines (p. 166, 23, 24) be studied further with a view to eliminating chance errors and that the modification described in the associate referee's report or some other modification of the Peynaud procedure be tested further.

(14) That the sulfite method for the determination of aldehydes in whiskey and other potable spirits be made tentative and that work on it be continued with a view to making it official.

(15) That study on the determination of total sulfur in wines be conducted.

(16) That the evaporation method described in the associate referee's report, and A.O.A.C. Method II (p. 167, 24)[2] for volatile acids in wines, be further studied to determine their applicability to distilled spirits, and that the cause of the slight loss resulting from the use of Method II be investigated.

(17) That the procedure for the quantitative determination of methanol in distilled spirits by the use of the neutral wedge photometer described in the associate referee's report be subjected to collaborative study for possible further improvement and simplification.

REPORT ON DIASTATIC ACTIVITY OF MALT

By CHRISTIAN RASK* (Albert Schwill & Co., Chicago, Ill.), *Associate Referee*

The method for the determination of diastatic power of malt adopted as tentative by this Association and also as the official method of the

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* Presented by J. A. LeClerc.

American Society of Brewing Chemists[1] is subject to much criticism because of the wide variations in results obtained in collaborative determinations.

According to Sallans and Anderson[2] the chief cause for the variations is the use of Fehling's solution for the titration of the converted starch solution. The method does not define in sufficient details the quantity of solution to be added for the preliminary boiling, neither does it specify the total boiling time, and it is doubtful whether it is at all possible to standardize a manipulation that would enable the individual analysts to perform the titration in identical manner.

Anderson and Sallans[3] developed a procedure based upon the present method of analysis with one exception—it depends upon the reduction of alkaline ferricyanide for the measurement of the converted starch. In a recent paper Laufer, Schwarz, and Laufer[4] recommended certain modifications of this method: the use of 1 cc. of malt infusion for malts of both low and high diastatic power, and a new basis for the calculation of maltose units and degrees Lintner.

During the past year the Associate Referee has studied the ferricyanide method outlined by Anderson and Sallans and the revised method developed by Laufer, Schwarz, and Laufer, and finds that more accurate results can be obtained with these than is possible with the methods based upon the titration with Fehling's solution.

Because the American Society of Brewing Chemists and also the Malt Analysis Standardization Committee of the American Association of Cereal Chemists have appointed special committees for the purpose of studying the numerous methods developed during recent years, no independent collaborative work has been done under the auspices of this Association.

The Associate Referee is in close cooperation with these two organizations, and in consideration of their current studies it is recommended that the present tentative method of this Association be retained as such for another year. It is expected that a method can be recommended at the next annual meeting and that it may be adopted as official before the publication of the fifth edition of *Methods of Analysis, A.O.A.C.*

No formal report on proteolytic activity of malt was given by the associate referee.

No report on malt extract in malt was given by the associate referee.

[1] *A.S.B.C. Official Methods*, 1937.
[2] *Cereal Chem.*, 14, 708 (1937).
[3] *Can. J. Research*, 15, 70 (1937).
[4] *Am. Brewer*, June, 1938.

REPORT ON MALT ADJUNCTS

By F. P. Siebel, Jr.* (J. E. Siebel Sons' Company, Chicago, Ill.),
Associate Referee

Methods for the analysis of malt adjuncts have been studied in great detail by the American Society of Brewing Chemists, and the Associate Referee has been in close contact with this work. Under the general heading of Methods for Analysis of Raw and Processed Cereals, the A.S.B.C. has adopted procedures with reference to Sampling, Physical Characteristics, Moisture, Oil, and Extract.

In the interest of avoiding needless duplication of efforts, close cooperation between the two organizations appears to be of great advantage to both. It is therefore recommended that the above identified methods of the A.S.B.C. receive consideration for adoption by the A.O.A.C. in the near future.

While extensive collaborative work has already been conducted on these methods by the A.S.B.C., all requirements demanded by the A.O.-A.C. will also be fulfilled. To this end, further collaboration is planned, not only among members of the A.S.B.C., but also among the collaborators on alcoholic beverages of the A.O.A.C. It is hoped that the progress of this work will be such that the methods can be recommended for adoption by the A.O.A.C. as tentative, and be included in the next printing of *Methods of Analysis*.

It is also anticipated that in the near future, further studies pertaining to malt adjuncts will suggest themselves. In particular it appears to be desirable to make a further study of the influence that differences of manufacture or processing of products such as grits and flakes have on the readiness with which their oil content yields to quantitative estimation, and on the advantages or disadvantages of substituting enzyme preparations for the ground malt in the determination of extract.

REPORT ON BEER

By Hugo W. Rohde* (Jos. Schlitz Brewing Company, Milwaukee, Wis.),
Associate Referee

In June, 1938, letters were addressed to thirty-three individuals, inviting them to participate in collaborative work on beer. Eighteen responded favorably, and to these people were sent five bottles of one beer and mimeographed copies of the methods of analysis to be used in making the determinations. Fourteen persons submitted the results of their findings, and these results are incorporated in this paper. To the

following collaborators who participated the Associate Referee wishes to express his appreciation.

Arthur E. Burhenn, Baltimore, Md.
D. Frederick Burnett, Newark, N. J.
N. J. Menard, Washington, D. C.
Morris A. Pozen, New York City
J. Bernard Robb, Richmond, Va.
Jos. Schlitz Brewing Company, Milwaukee, Wis.
Schwarz Laboratories, New York City
E. A. Siebel and Company, Chicago, Ill.
Stephl Laboratories, Milwaukee, Wis.
Wahl-Henius Institute, Chicago, Ill.
J. E. Siebel Sons' Company, Chicago, Ill.
Wallerstein Laboratories, New York City
J. B. Wilson, Washington, D. C.

Some of the collaborators submitted data of determinations made in duplicate and triplicate, and a number did not complete all the determinations outlined owing to lack of the necessary apparatus.

COMPARISON OF RESULTS

Close agreement, within the error of experiment, is shown in the data submitted by laboratories in which beer analysis is a daily routine. The following comments relate to the results obtained, and they follow the order given on the sheets sent to the collaborators.

Apparent Extract.—This was obtained by taking the specific gravity of the decarbonated beer at 20°/20° C., either with the Reischauer pycnometer, or with the Boot vacuum jacketed pycnometer (the latter type preferred) and obtaining the corresponding values from the Plato tables. Seventeen results ranged between 3.26 per cent and 3.54 per cent, averaging 3.40 per cent. Twelve results were within 0.05 per cent (plus or minus) of the average.

Alcohol (Per Cent by Weight).—Seventeen results ranged from 3.54 to 3.85 per cent by weight, the average being 3.72 per cent. Data from nine brewing laboratories varied from 3.64 to 3.80 per cent, averaging 3.71 per cent.

Real Extract.—The results of seventeen determinations ranged from 4.51 to 5.29 per cent. If the three lowest figures are omitted, the average is 5.15 per cent. Nine determinations were within 0.05 per cent (plus or minus) of the average.

Extract of Original Wort.—This is the concentration of the malt liquor before fermentation. The original formula, according to which this calculation was made, was devised by Carl Balling several generations ago, and is still considered to be quite accurate. It is based upon the amount of alcohol formed in fermentation, the unfermented extract present in the beer, the carbon dioxide produced, and the yeast formed during the fermentation of the wort. Later investigations have shown that the value

of the yeast multiplication is too high. The original extract of the wort can readily be calculated from the values of the alcoholic content and the real extract of the beer, namely:

2 Alcohol + Extract ± Correction.

For the average American beer the product of the alcohol by weight times 1.93 plus the real extract gives the original extract of the wort. If the latter computation is used in connection with the beers here given, the values of the original extract varies but 0.05 per cent in most of the cases from the values calculated according to the original formula.

The minimum for the original extract was 11.7 per cent; the maximum, 12.5 per cent; and the average 12.1 per cent.

Real and Apparent Degrees of Fermentation.—These values are computed for the real and apparent extracts and the original extract of the wort. The data in these tables show fairly close results.

Real Extract: 55.0–62.1 per cent, average 58.2 per cent.

Apparent Extract: 70.7–72.6 per cent, average 71.8 per cent.

Ash.—Seventeen results ranged from 0.13 per cent to 0.17 per cent. If the only high value is omitted, the average of sixteen determinations is 0.14 per cent.

Phosphoric Acid (P_2O_5).—Twelve data were submitted, varying from 0.026 per cent to 0.050 per cent; eleven data were between 0.045 per cent and 0.050 per cent, averaging 0.047 per cent. This determination was made according to the official A.O.A.C. method. One collaborator commented on this method, stating that the results obtained were too low, and that he preferred the gravimetric method.

Reducing Sugars.—The official method was used, and fourteen determinations varied from 1.19 per cent to 1.38 per cent, the average being 1.30 per cent when the two lowest figures are omitted.

Dextrin.—Twelve determinations for dextrin gave a minimum of 2.44 per cent, and a maximum of 2.84 per cent, averaging 2.66 per cent.

Protein.—With one exception, the figures of eleven protein determinations ranged between 0.33 per cent and 0.39 per cent, averaging 0.34 per cent.

Iodine Reaction.—This determination is made in order to ascertain whether or not the carbohydrates of the malt-wort have been properly converted. The test is subjective, and depends largely upon the observer. Five collaborators reported no reaction with iodine, and six reported an erythrodextrin (reddish coloration) reaction. From a technical viewpoint only a blue or violet coloration is objectionable.

Carbon Dioxide.—A method for the determination of carbon dioxide in beer has been developed by Philip P. Gray of the Wallerstein Laboratories. This method was submitted to the collaborators and six made eleven determinations. The minimum found was 0.42 per cent of CO_2 by weight, the maximum, 0.51 per cent, and the average, 0.46 per cent. This

Collaborative results on beer

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Specific gravity (20°/20° C.)*	1.01363 / 1.01292	1.01322	1.01330	1.0130	1.01373	1.01336	1.01346	1.01336	1.01330	1.01330	1.01352	1.01358	1.01347	1.01332
Apparent extract (%)	3.48 / 3.30	3.38	3.40	3.26	3.54	3.41	3.44	3.42	3.39	3.40	3.45	3.44	3.50	3.40
Alcohol (% by weight)	3.43 / 3.85	3.84	3.78	3.85	3.54	3.64	3.73	3.80	3.75	3.78	3.66	3.70	3.65	3.68
Alcohol (% by volume)	4.56 / 4.92	4.91	4.85	4.94	4.45	4.67	4.79	4.88	4.80	4.78	4.70	4.65	4.60	4.63
Real extract	5.18 / 5.27	5.29	4.89	4.51	5.23	5.13	5.12	5.13	5.12	5.14	5.13	5.17	5.10	5.07
Reducing sugars, maltose (per 100 ml.)*	1.33 / 1.27	1.27	1.25	—	—	1.30	1.33	1.20	1.31	1.28	1.31	1.32	1.34 / 1.19	1.38
Dextrin (%)	2.72 / 2.68	—	2.46	—	2.71	—	2.74	2.84	2.73	2.62	2.44	2.65	2.60	2.82
Protein (N×6.25) (%)	0.51	—	0.34	—	—	0.34	0.33	0.39	0.34	0.34	0.32	0.35	0.35	0.34
Ash (%)	0.14	0.15	0.15	0.14	0.14	0.14	0.14	0.17	0.14	0.14	0.13	0.14	0.14	0.14
Phosphoric acid (P_2O_5) (%)	0.044 / 0.046	0.047	0.048	—	—	0.045	0.050	0.026	0.050	—	0.046	0.048	0.046	—
Total acidity as lactic acid (%)	0.15 / 0.14	—	0.13	—	0.14	0.10	0.14	0.11	0.13	0.14	0.15	0.14	0.13	0.13
Carbon dioxide (% by weight)	0.42, 0.44 / 0.47, 0.51	—	—	—	—	0.48	0.47, 0.45, 0.47	—	0.46	0.49	0.46	0.47, 0.50	—	0.13
Carbon dioxide (% by volume)	2.17, 2.26	—	—	—	—	2.45	2.42, 2.32, 2.42	—	2.35	2.51	2.35	2.42, 2.55	—	—
pH value	4.40	4.40	4.24	—	Erythro	4.40	4.35	4.55	4.40	4.45	4.43	4.44	4.50	4.42
Iodine reaction	0	—	0	—	Erythro	Erythro	0	0	Erythro	0	0	Erythro	Erythro	Erythro
Sulfur dioxide (SO_2) (mg. per l.)	4.10	4.10	5.48	—	—	3.4	7.6	3.3	5.0	—	14.9	5.8	—	—
Iron (Fe) (p. p. m.)	0.20	0.20	0	—	—	0	<0.5	0.55	0.6	<0.2	0.3	0.25	0.25	—
Color (Lovibond S. 52, 1/2" Cell)	—	—	—	—	—	3.7	4.3	3.0	4.5	4.3	4.25	3.75	4.3	4.25
Calculated data:														
Original extract (%)	11.9 / 11.7	11.7	12.1	11.9	12.1	12.2	12.3	12.4	12.4	12.5	12.2	12.4	12.1	12.2
Real Degree of Fermentation (%)	57.4 / 55.0	56.6	59.5	62.1	61.9	56.7	58.0	58.5	58.6	58.8	58.0	57.9	57.8	58.4
Apparent degree of fermentation (%)	70.8 / 71.2	71.1	71.9	72.6	71.7	70.7	72.0	72.4	72.6	72.8	71.7	72.1	71.0	72.1

* Official method.

corresponds to 2.17–2.66 per cent by volume. The average is 2.38 per cent by volume. It happens occasionally that the closure on the bottle is not perfectly tight, and a loss of gas results. The bottles sent out for analysis were carefully examined in this respect.

Color.—The determination of color is a subjective matter. Tests have shown that the glasses of the Lovibond tinctometer are not absolutely correct. The color ranged from 3.0 to 4.5. If the lowest value is omitted, the average is 4.17.

Acidity.—The total acidity of thirteen determinations reported ranged from 0.10 per cent to 0.15 per cent, averaging 0.13 per cent. Omitting the two lowest results, eleven determinations averaged 0.14 per cent.

Hydrogen Ion Concentration or pH Value.—Twelve collaborators reported results varying from 4.24 to 4.55, and averaging 4.35.

Sulfur Dioxide (SO_2).—Seven determinations averaged 5 mg. of SO_2 per liter, ranging from 3.4 to 7.49, the highest figure being excluded. One collaborator stated that collecting 70 cc. of distillate from 200 cc. of beer is insufficient for this determination.

Iron.—Iron dissolved in beer and present in more than appreciable quantities will impair its durability. The colorimetric determination made by eleven analysts gave 0–0.6 p.p.m.

CONCLUSIONS

The fifteen determinations outlined in this work cover practically all the analytical tests required for judging the quality of a beer. In addition, a microscopic and bacteriological examination and a determination of dissolved air may be necessary. The practice of using antiseptics is no longer resorted to as modern brewery equipment and proper supervision have made this practice obsolete.

Methods of Analysis, A.O.A.C., 1935, in Chapter XIV on "Malt Beverages, Sirups and Extracts, and Brewing Material" contains: Par. 11, Volatile Acids; 14, Direct Polarization; 15, Glycerin; 27, Chlorides; and 28, Methyl Alcohol. These may all be omitted in future editions.

The American Society of Brewing Chemists has been actively engaged for several years in formulating methods for the analysis of beer. At the May, 1939, meeting, no doubt action will be taken on the adoption of certain methods for analysis that are now being studied. This being the case, the Associate Referee will refrain from making suggestions as to recommendations[1] in connection with the analysis of beer at this time.

No report on heavy metals in beer was given by the associate referee.

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[1] For report of Subcommittee D and action by the Association, see *This Journal*, **22**, 65 (1939).

REPORT ON CARBON DIOXIDE IN BEER

By P. P. GRAY (Wallerstein Laboratories, New York City),
Associate Referee

The tentative A.O.A.C. method for the determination of carbon dioxide in beer, *Methods of Analysis, A.O.A.C.*, 1935, 151, is somewhat time-consuming. A shorter method, suitable for laboratories equipped with the necessary apparatus, is the pressure-air method recently described by Gray and Stone.[1]

Last year it was decided to compare the two methods, and accordingly collaborative work by seven different laboratories was conducted. Two samples, representing beer of two different air contents, were sent to the collaborators. The air content of Sample No. 1 varied from 1.5 to 3.0 cc. per bottle, while the No. 2 beer contained from 6 to 10 cc. per bottle. The CO_2 results are given in Table 1.

TABLE 1.—*Collaborative results on CO_2*

(Results expressed as per cent)

COLLABORATOR		1	2	3	4	5	6	7	GRAND AVERAGE
Sample No. 1									
A. Chemical		0.482	0.471	0.43	0.423	0.504	0.45	0.478	
Method		0.485	0.443	0.45	0.383*		0.46	0.480	
		0.483	0.436	0.46	0.467		0.46	0.477	
	Average:	0.483	0.450	0.45	0.445	0.504	0.457	0.478	0 467
B. Pressure		0.46	0.473	0.47	0.491	0.45	0.46	0.475	
Method		0.47	0.503	0.44	0.481	0.45	0.46	0.479	
		0.47	0.474	0.42	0.466		0.45		
	Average:	0.47	0.483	0.44	0.479	0.45	0.457	0.477	0.465
Sample No. 2									
A. Chemical		0.365	0.336	0.34	0.302	0.335	0.34	0.359	
Method		0.366	0.350	0.34	0.280	0.361	0.34	0.360	
		0.368	0.345	0.34	0.311		0.35	0.358	
	Average:	0.366	0.344	0.34	0.298	0.348	0.343	0.359	0.343
B. Pressure		0.37	0.314	0.35	0.341	0.32	0.34	0.357	
Method		0.36	0.368	0.33	0.343	0.34	0.34	0.356	
		0.36	0.368	0.35	0.344		0.34	0.334	
	Average:	0.36	0.350	0.34	0.343	0.33	0.34	0.349	0.345

* Not included in averages.

Owing to the simplicity of the pressure method, most brewing laboratories prefer it to the chemical method. As a result, few of the collaborating laboratories have had much experience with the chemical method. This fact probably accounts for some of the variations apparent in the tabulated results.

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[1] *Ind. Eng. Chem. Anal. Ed.*, **10**, 15 (1938).

Where the method had been used to any extent much better agreement was found to be the rule, for example in the results obtained in the Associate Referee's laboratories (Column 1). However, the results of the individual collaborators show a generally good agreement, and only minor variations are shown in the results of all the collaborators. In general sufficiently good agreement is apparent in the pressure method results to warrant recommendations that this method be adopted as a tentative method.

The complete directions for the pressure-air method, which was adopted as tentative, follow. Inadvertently the first part of the method was omitted when it was published in *This Journal*, **22**, 73 (1939).

CARBON DIOXIDE IN BEER

Pressure Air Method

APPARATUS

Piercing apparatus.—A gas tight packing box and fastening for adjustment over the crown of the bottle, which holds a hollow spike. (A suitable apparatus may be obtained from a number of manufacturers.) With a can a metal frame, the top of which is pressed or screwed down and locked over the can top, holds a hollow spike surrounded by a compressible rubber sealing plug. The spike leads to an accurate pressure gage and an outlet valve.

One apparatus, adjustable for bottles and cans, may be used.

Absorption buret.—The buret as shown in Figure 1 consists of a graduated tube (0–6 cc. graduated in 0.1 cc. divisions and 6–25 cc. graduated in 0.2 cc. divisions), having a bulb, and closed at each end by stop cocks, The upper end is connected by rubber tubing to the outlet valve of the piercing apparatus and the lower end is connected by a length of rubber tubing to a leveling bulb.

DETERMINATION

If the sample is in a bottle, make a scratch mark at the beer level; if the sample is in a can, weigh the can with the contents. Submerge the container in a water bath at 25°C. long enough to bring the temperature of the beer to 25°C. Connect the piercing apparatus to the bottle or can. Fill the absorption buret with 15% NaOH solution and allow the solution to run up to stopcock B. Fill the upper capillary of the absorption bulb with hexyl alcohol and the remainder of the system between B and the tip of the spike with water in order to displace any air. With outlet valve A closed, drive the spike through the crown or can top and thoroughly shake and tap the bottle or can. Make pressure reading on the gage. Again shake and take pressure readings. Use the pressure reading that shows no change in consecutive readings.

Open stopcocks B and C of the absorption buret and then outlet valve A. Allow the gas, together with foam, to flow over into the absorption buret. Swirl contents of the buret to permit absorption of CO_2. When one-half to three-fourths of the alkali solution in the absorption buret has been displaced, shut off all the stopcocks and shake to permit complete absorption of CO_2. Set the buret in a vertical position, open the bottom stopcock, C, and allow alkali to flow back into the bulb. Open stopcocks B and A and repeat the above operation, tapping the bottle or can to accelerate evolution of CO_2. Close the upper stopcocks A and B and shake thoroughly to absorb the last traces of CO_2. Bring the leveling bulb to such a position that the height of the solution in the leveling bulb and buret are the same and

read the unabsorbed gas, which is reported as "air." Repeat the operations until consecutive readings as to "air" are the same.
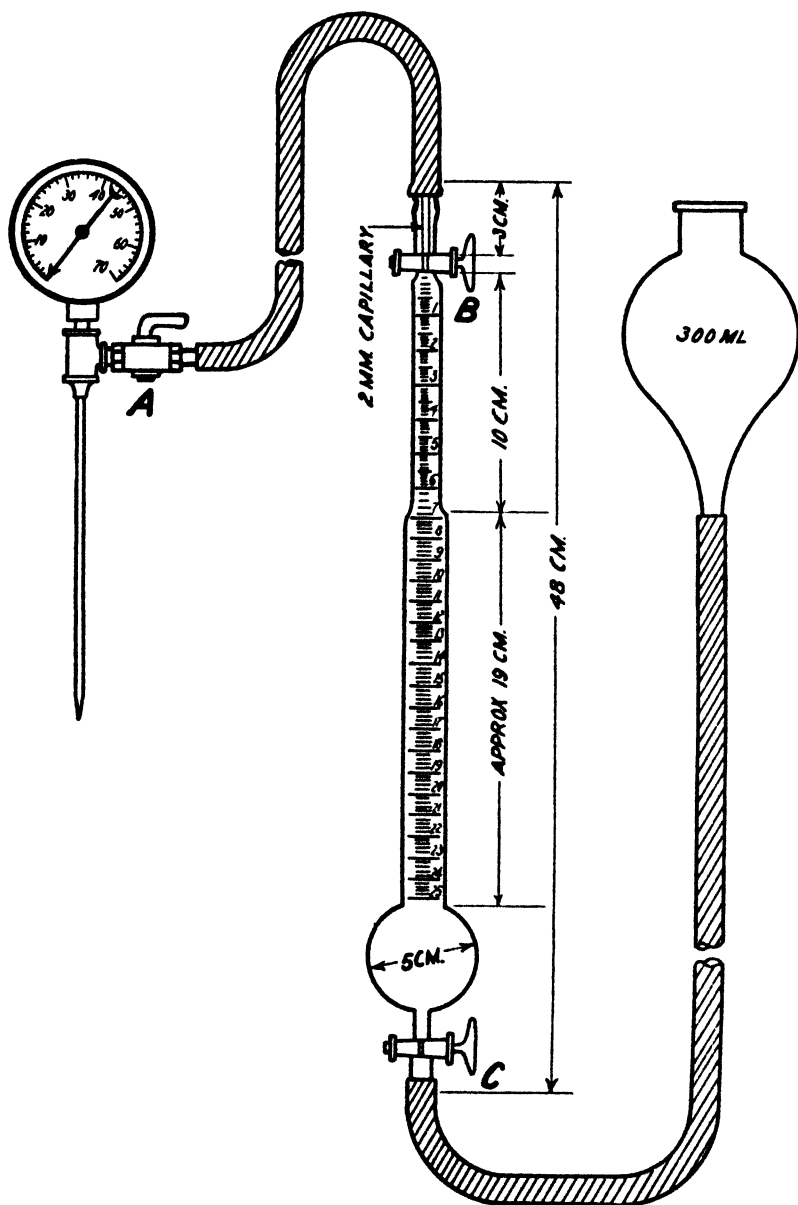
FIG. 1.—ABSORPTION BURET

Disconnect the bottle or can and determine the head-space volume as follows:
If the sample is a bottle, fill with water to the top and pour off into a graduated cylinder to the scratch mark. The number of cc. of water thus poured off represents head space in cc.

If the sample is a can, weigh empty can after pouring out all remaining beer. The difference represents the weight of beer, which divided by the sp. gr. of the beer will give volume of beer in cc. Fill the empty can with water and weigh. Weight of water in grams is also the volume in cc., so that the difference between volume of water and volume of beer represents head space in cc.

Calculate CO_2 by weight by the following formula:

$$\%CO_2 = \left[P - \left(\frac{cc.\ of\ air}{cc.\ of\ head\ space} \times 14.7 \right) \right] \times 0.00965, \text{ in which}$$

P = absolute pressure in pounds per sq. in. at 25° C. = (ordinary gage pressure +14.7). (For routine work 15 may conveniently be substituted for 14.7.)

It is recommended that the pressure-air method for the determination of carbon dioxide in beer be adopted as a tentative method and that study of the method be continued.

REPORT ON WINES

By B. G. HARTMANN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

No work was done on total sulfur in wines.

Sufficient work was done on the saponfication of esters with lead acetate to show that acid ethyl tartrate is not saponified by treatment with lead acetate solution. The reaction in the case of diethyl tartrate proceeds rapidly, but the second ethyl group remains untouched even on continued heating. Since the esters in wines exist in the acid form, it is evident that for the purpose of their saponification treatment with lead acetate is ineffective.

RECOMMENDATIONS[1]

It is recommended—

(1) That the study on the determination of total sulfur be continued.

(2) That the study of the saponification of esters with lead acetate be dropped.

REPORT ON VOLATILE ACIDS IN WINE

By M. A. JOSLYN, (University of California, Berkeley, Calif.), *Associate Referee*

In the first report by this Associate Referee, *This Journal*, 21, 166 (1938), it was pointed out that the distillation methods used at present yield results that are reproducible by different chemists to not better than ±0.006 gram of acetic acid per 100 cc. on the average (corresponding to a titration error of 0.1 cc. of 0.1 N base per 10 cc.

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aliquot of wine). Unexplainably large departures from this mode often occurred, particularly with dry red wine of fairly high volatile acidity.

To obtain more information as to sources of these variations the following six samples were sent out for analysis on September 1, 1937:

SAMPLE	DESCRIPTION	SP. GR. 20/20	ALCOHOL	TOTAL ACIDITY AS TARTARIC
A	Solution of acetic acid in water	—	—	0.121*
B	Synthetic wine containing per 100 cc., approx. 0.10 g. of acetic acid, 5 g. of dextrose, 0.4 g. of malic acid, 0.3 g. of cream of tartar, and 13% of alcohol	1.200	12.6	0.648
C	Dry white wine	1.190	14.5	0.706
D	Dry red wine	1.309	14.8	0.750
E	Port type	1.466	20.3	0.735
F	Sherry type	1.084	21.0	0.550

* 0.097 gram of acetic acid per 100 cc.

The wines were thoroughly mixed and then filled into 6 oz. crown-capped bottles. The bottles were filled completely to avoid changes in the volatile acidity, particularly in the artificial wines. Samples were sent to the same thirty collaborators, but results were obtained only from the twelve mentioned. The date of the analysis is given because it was found that Sample D became infected with acid-producing organisms.

COLLABORATORS

Steward Berkshire, Deputy Commissioner of Internal Revenue, U. S. Treasury Department, Washington, Reported October 2, 1937.

Lyman Cash, Chemist, B. Cribari and Sons, Madrone, Calif. Reported September 17, 1937.

J. M. Curtis and Son, Wine Analysts for California Department of Health, Bureau of Food and Drug Inspection (through Milton P. Duffy, chief). Reported October 6, 1937.

Edwin N. Davis, Junior Chemist, Food Research Division, U. S. Bureau of Chemistry and Soils. Reported November 29, 1937.

Ray Dunn, Laboratory Assistant, Fruit Products Division, University of California (now with State Department of Chemistry at Sacramento, Calif.). Analyzed in October.

Robert A. Greene, Director, Arizona State Laboratory, Tucson. Reported October 13, 1937.

R. F. Love, Field Chemist, Internal Revenue Service, U. S. Treasury Department, San Francisco. Reported September 18, 1937.

Anna E. Mix, Beverage Section, Food Division, U. S. Food and Drug Administration. Analyzed November 1937.

J. Bernard Robb, Chemical Director, Virginia Alcoholic Beverage Control Board, Richmond. Reported October 11, 1937.

Bertha Schwartz, Schenley Products Company, New York City. Reported October 1, 1937.

T. E. Twining, The Twining Laboratories, Fresno, Calif. Reported September 15, 1937.

TABLE 1.—Results (expressed as gram of acetic acid per 100 cc.) on volatile acidity of acetic acid solutions and wines obtained by several specified methods

COLLABORATOR	A (ACETIC ACID)				B (SYNTHETIC WINE)				C (DRY WHITE)			
	1	2	3	4	1	2	3	4	1	2	3	4
S. Berkshire	0.090	0.084	0.084	0.088	0.095	0.090	0.096	0.096	0.095	0.090	0.093	0.090
L. Cash	0.080	0.087	0.084	0.087	0.084	0.096	0.096	0.096	0.085	0.102	0.102	0.099
J. Curtis	0.091	0.090	0.090	0.092	0.094	0.096	0.093	0.096	0.096	0.084	0.084	0.089
E. N. Davis	0.079	0.084	—	0.092	—	0.092	—	0.096	0.062	0.085	—	0.093
R. Dunn	—	0.092	0.092	0.092	—	0.094	0.098	0.095	—	0.092	0.091	0.093
R. A. Greene	0.085	0.076	—	0.085	0.085	0.085	—	0.085	0.085	0.079	—	0.085
R. F. Love	0.089	0.090	0.084	0.087	0.090	0.096	0.090	0.093	0.094	0.091	0.087	0.090
Anna E. Mix	0.085	0.083	0.085	0.079	0.090	0.096	0.085	0.079	0.092	0.090	0.087	0.090
J. B. Robb	0.089	0.093	0.090	0.084	0.092	0.135*	0.090	0.093	0.096	0.099	0.097	0.084
Bertha Schwartz	0.061	0.070	0.079	0.066	0.061	0.097	0.102	0.072	0.068	0.076	0.074	0.061
T. E. Twining	0.085	0.081	0.091	0.077	0.092	0.099	0.098	0.097	0.091	0.085	0.087	0.074
J. L. Weinberg	0.088	0.086	0.091	0.086	0.090	0.091	0.091	0.094	0.082	0.083	0.086	0.086
M. A. Joslyn*	—	—	0.089	—	—	—	0.084	—	—	—	0.084	—
Average	0.084	0.0845	0.087	0.0845	0.087	0.093	0.094	0.091	0.086	0.088	0.089	0.086
Maximum	0.091	0.093	0.092	0.092	0.095	0.097	0.102	0.097	0.096	0.102	0.102	0.099
Minimum	0.061	0.070	0.084	0.066	0.061	0.085	0.085	0.072	0.062	0.076	0.084	0.061

* Not averaged.

COLLABORATOR	D (DRY RED)				E (PORT)				F (SHERRY)			
	1	2	3	4	1	2	3	4	1	2	3	4
S. Berkshire	0.124	0.118	0.120	0.120	0.083	0.078	0.084	0.084	0.079	0.081	0.081	0.082
L. Cash	0.112	0.141	0.126	0.126	0.074	0.087	0.081	0.081	0.074	0.081	0.084	0.084
J. Curtis	0.127	0.117	0.117	0.117	0.081	0.075	0.057	0.075	0.080	0.078	0.054	0.078
E. N. Davis	0.092	0.120	—	0.123	0.069	0.075	—	0.081	0.058	0.078	—	0.079
R. Dunn	—	0.123	0.122	0.123	—	0.082	0.084	0.079	—	0.079	0.081	0.078
R. A. Greene	0.148	0.120	—	0.169	0.100	0.073	—	0.076	0.082	0.076	—	0.076
R. F. Love	0.121	0.120	0.120	0.108	0.077	0.084	0.081	0.078	0.072	0.075	0.073	0.077
Anna E. Mix	0.119	0.119	0.115	0.117	0.077	0.072	0.078	0.078	0.070	0.076	0.078	0.083
J. B. Robb	0.126	0.192	0.117	0.123	0.077	0.114	0.078	0.078	0.073	0.075	0.078	0.078
Bertha Schwartz	0.070	0.072	0.100	0.076	0.054	0.069	0.074	0.059	0.051	0.071	0.077	0.066
T. E. Twining	0.117	0.128	0.134	0.126	0.078	0.083	0.091	0.079	0.079	0.084	0.092	0.081
J. L. Weinberg	0.104	0.103	0.108	0.108	0.074	0.074	0.074	0.077	0.084	0.086	0.091	0.086
M. A. Joslyn*	—	—	—	—	0.074	—	0.082	—	—	0.072	—	—
Average	0.114	0.123	0.118	0.120	0.077	0.081	0.078	0.077	0.073	0.078	0.077	0.079
Maximum	0.148	0.192	0.134	0.169	0.100	0.114	0.091	0.084	0.084	0.086	0.092	0.086
Minimum	0.092	0.072	0.100	0.108	0.054	0.069	0.057	0.059	0.051	0.075	0.054	0.066

Jesse L. Weinberg, Chemist, Polak's Frutal Works, Inc., Long Island City. Reported October 26, 1937.

It was requested that the volatile acidity of the samples be determined by the following methods:

1. Method I, Official A.O.A.C. procedure (50 cc. of wine steam distilled). *Methods of Analysis*, 1935, p. 166.

2. Method II, Official A.O.A.C. procedure (10 cc. steam distilled). *Methods of Analysis*, 1935, p. 167.

3. With apparatus used in Method II, but preferably with a vertical condenser. Adjust the flow of water in condenser so that distillate comes over hot (about 70° C.), collect 100 cc. of distillate, and titrate hot.

4. As in Method II but collect 100 cc. of distillate, bring to boil, and titrate hot.

The results obtained are summarized in Table 1, as are also more recent analyses (October 1938) obtained by the Associate Referee. At this date the total titratable acidity in A expressed as acetic acid was 0.093 gram per 100 cc., a slight decrease from the previous value of 0.097. Sample D was gassy and had increased to 0.380 gram of acetic per 100 cc. There is considerable variation in the results reported, particularly with the dry red wine. The results reported by Bertha Schwartz are particularly low, and this collaborator obtained higher values throughout by Method 3. Twining obtained higher results by Method 3 and considerably lower results by Method 4, although this trend is not shown in the results obtained by others. There is too much variation in the results to show any definite trend in favor of any method. The most erratic results were obtained with the dry red wine. Changes in volatile acidity during the time that elapsed between shipment of samples and analysis probably does not account for this variation. No significant changes in volatile acidity of the samples of dry red wine occurred during a period of two months. However, the highest acidity was reported by Collaborators Greene and Robb. The latter analyzed the samples early in October, and although Davis reported late in November he stored his samples at 35° F.

From the data available it is difficult to determine whether the variations in results are due to erratic errors or whether systematic errors were coming in. The Associate Referee's results indicate that when the conditions of distillation are properly controlled, results may be readily duplicated to within a titration error of ±0.02 cc. Thus Ray Dunn obtained the results shown in Table 2. He used the C. H. McCharles still (also known as B-K-H still), which consists of a large Sellier tube sealed into an Erlenmeyer flask and in which the 10 cc. of wine is steam distilled over an electric plate.

A comparison of the modified Fessler apparatus, the McCharles still, and the large Sellier tube official apparatus was made by Dunn to determine effect of variation in type of still. However his results, Table 3,

TABLE 2.—*Results obtained by Ray Dunn*

WINE	VOLUME DISTILLED (CC.)						TOTAL TITRA-TION, CC. 0.1 N NaOH
	50	10	10	10	10	10	
	cc. 0.1 N NaOH REQUIRED FOR EACH ALIQUOT						
A	1.36	0.07	0.05	0.03	0.03	0.02	1.55
	1.31	0.10	0.05	0.03	0.02		1.51
B	1.28	0.12	0.05	0.06	0.03	0.02	1.56
	1.35	0.10	0.04	0.04	0.04	0.02	1.58
	1.28	0.10	0.05	0.05	0.03	0.02	1.53
C	1.28	0.08	0.06	0.07	0.04	0.05	1.58
	1.28	0.06	0.08	0.07	0.05	0.03	1.57
D	1.67	0.10	0.10	0.08	0.05	0.05	2.05
	1.69	0.09	0.09	0.09	0.04	0.05	2.05
E	1.10	0.08	0.04	0.05	0.03	0.03	1.33
	1.17	0.06	0.05	0.05	0.03	0.03	1.39
F	1.05	0.08	0.06	0.05	0.04	0.03	1.31
	1.05	0.08	0.05	0.05	0.04	0.04	1.31

do not indicate that variation in the type of apparatus used had any significant effect.

TABLE 3.—*Effect of variation in type of apparatus*
(Results by Dunn)

WINE	FESSLER	MCCHARLES	LARGE SELLIER
A	0.092	0.092	0.092
B	0.095	0.094	0.098
C	0.093	0.094	0.091
D	0.123	0.123	0.123
E	0.079	0.082	0.084
F	0.078	0.079	0.081

Several of the collaborators experimented with various phases of the determination, and the results of these experiments together with pertinent comments are given as follows:

E. N. Davis.—In addition to the methods listed, results were obtained by Method 5, in which a Braun-Knecht-Heimann apparatus was used with 20 cc. of sample, the end point being taken where 10 cc. distillate is required to neutralize two drops of 0.1 N alkali. The results obtained with this method, expressed as gram of acetic acid per 100 cc., are as follows:
A—0.087, B—0.088, C—0.074, D—0.119, E—0.076 and F—0.068.

Davis commented on Method 2 as follows:

It was found that in bringing over the distillate at 60° C. or above, a considerable amount of the distillate was not condensed, and therefore a considerable part of the volatile acids was lost. Further, under the usual circumstances in the laboratory, e.g., varying water pressure, the temperature in the condenser could not be held constant with the set-up specified so that the use of this method was impractical.

The following additional comments were added:

1. In order to check on the efficiency of various methods of CO_2 removal from the titration, a sample of sparkling wine was used with Method 2 and various modifications, i.e., (a) incipient boiling, then cooling before sample is taken, (A.O.A.C.); (b) sample heated in the Sellier tube in boiling water bath until alcohol began to be evaported (90° C.)—this was before the tube was connected to the distillation trap and condenser; (c) sample taken after part of CO_2 was removed by pouring back and forth from beakers; (d) with as little removal of CO_2 as possible before the sample was distilled.

Results:	a	b	c	d
Standard alkali (cc.)	1.48 and 1.53	1.53	1.48 and 1.50	1.48

Modification (b) is, in my opinion, the most practical and time-saving method. Although it would seem that CO_2 does not interfere seriously, the close checks should not be taken at their face value as I know that under certain conditions the fading end point due to CO_2 in the distillate may give a high value.

2. More than the 100 cc. of water in the jacket called for in A.O.A.C. Method II is necessary as the sample may be superheated when the water level is lowered. This higher temperature forces over "fixed" acids. 150 cc. of water usually suffices.

3. Neither paraffin nor other foam-reducers are adequate with some wines, especially young dry wines with high protein content. If the distillation trap is large enough (2½" d. bulb) and the trap outlet is large enough so that the condensed distillate may run back, no foam reducer is usually necessary although it may be necessary to cut down the rate of distillation with the worst wines. I have not attempted to determine whether the rate of distillation is a function of the end point, although I have seen some evidence to that effect.

Regular evolution of water vapor into the wine is important with badly foaming wines and a few grains of granulated Zn in the water jacket has proved satisfactory in preventing bumping.

4. The total titration requires so little alkali (2 cc. of 0.1 N is the legal limit) that serious percentage errors may occur. The use of a 20 cc. sample as in the B-K-H apparatus will reduce the error by one-half if it is remembered that the definition of the end point must be changed. It might also be worth while to define the end point in terms of size of sample taken when a 0.02 N alkali solution is used. For a 10 cc. sample, it would probably be "tritrate each succeeding 10 cc. of distillate until *5 drops* of 0.02 N alkali are required tor each the neutral point." The use of 0.02 N alkali would probably give a more sensitive end point in addition to using five times the amount of alkali.

It might also be worth while to check the recovery of volatile acids at different concentrations, especially between 0.1 and 0.015 per cent. This could be done by adding known amounts of acetic acid (or a mixture of appropriate volatile acids and esters) to *wines* (not water) of known volatile acid content. This should throw some light on the absolute efficiency of the methods.

S. Berkshire.—Considerable difficulty was experienced with Method 3, due to the

difficulty of controlling the temperature of the water in the condenser. Of the methods used, No. 4 seemed preferable.

R. F. Love.—Methods 1, 2, 3, and 4 are in accordance with your letter. In addition to the four methods requested we determined the volatile acids in accordance with the method usually followed in this laboratory—listed as Method 5 in the report. (The results obtained from Method 5, expressed as gram of acetic acid per 100 cc., are as follows: A—0.090, B—0.093, C—0.093, D—0.123, E—0.084, F—0.078.)

Method 1: We are of the opinion that this method is open to criticism due to the fact that steam distillation is discontinued before all the volatile acids are removed, although repeated titrations of 15 cc. portions of the distillate tend to introduce a compensating error.

Method 2: It is believed that this method is satisfactory although a small error may be introduced by the titration of several small portions of the distillate. We believe that it is preferable to collect 100 cc. of distillate and titrate it in the case of ordinary wines such as those submitted. We believe it unnecessary to bring this small sample to boiling before steam distilling it as the amount of CO_2 in the 10 cc. sample is negligible. In the case of sour wines our experience indicates that approximately 200 cc. of sample should be distilled before titration.

Method 3: Considerable difficulty was experienced in adjusting the flow of water in the condenser so that the distillate came over hot (about 70° C.). In running wines according to this method we believe it is almost impossible, using the ordinary condenser, to prevent the distillate from becoming too hot, with the resultant loss of volatile acids.

Method 4: It is believed that boiling the distillate will cause more or less loss of volatile acids, thus introducing a small error in the final figures.

Method 5: This method is the same as Method 2 except that the sample of wine is not brought to the boiling point prior to distillation, and 100 cc. of the distillate is collected and titrated. In the case of sour wines and vinegars a 5 cc. sample is distilled to 200 cc. and titrated. Our experience shows that the amount of distillate collected is sufficient to ensure the removal of the volatile acids from the sample.

J. Bernard Robb.—Following are the volatile acid results, expressed as gram of acetic acid per 100 cc., on the samples of wine sent us recently:

Samples	1	2 Round Bulb (Official)	2 Long Bulb (Virginia)	3	4	Va.
A	0.089	0.093	0.087	0.090	0.084	0.087
B	.0924	.135	.093	.090	.093	.093
C	.096	.099	.097	.084	.096	.096
D	.126	.192	.126	.117	.123	.123
E	.0768	.114	.078	.078	.078	.081
F	.0726	.075	.072	.078	.078	.081

In Method 2 we found we could not get accurate results on all analyses with the round bulb as shown in the official method, so substituted our bulb, which is a long bulb with a break in the main tube about midway. The results in the column marked "Virginia" were determined by our regular method and the long bulb, distilled fast with perpendicular condensers. The results in Column 2 (Round Bulb), slow for reasons stated, should be discarded; that is, the results on B, D, and E.

Bertha Schwartz.—My observations of the results lead me to suggest that greater control should have been used relative to the atmospheric conditions of the experiments. In Methods 2, 3, and 4 where only 10 cc. of the wine is used and its volatile

acidity is very low, the results varied somewhat with the temperature of the liquid when pipetting. The lower the atmospheric temperature the greater the amount of volatile acidity found by these three methods.

TABLE 4.—*Effect of alcohol on rate of distillation of acetic acid*

VOLUME DISTILLED	ALCOHOL CONTENT (% BY VOLUME)				
	0	5	10	15	20
	PER CENT OF TOTAL ACID PRESENT DISTILLED OVER				
cc.					
10	7.3	4.2	3.0	2.5	2.1
20	10.5	9.1	7.2	6.2	5.1
30	15.7	14.2	12.3	10.8	9.0
40	20.6	18.2	17.3	15.9	13.7
50	25.0	23.6	22.0	20.8	18.8
60	29.1	28.0	26.7	25.6	23.8
70	33.1	32.0	31.1	30.0	28.5
80	36.7	35.9	35.1	34.3	32.8
90	40.3	39.5	38.7	38.3	37.0
100	43.6	43.0	42.3	42.0	40.8
150	57.6	57.4	56.7	57.2	57.0

TABLE 5.—*Effect of sugar on rate of distillation of acetic acid*

VOLUME DISTILLED	PER CENT OF SUGAR			
	0	2	5	10
	PER CENT OF TOTAL ACID PRESENT DISTILLED OVER			
cc.				
10	7.3	5.4	5.9	5.3
20	10.5	10.7	11.8	11.2
30	15.7	15.7	17.3	17.0
40	20.6	20.6	22.7	21.9
50	25.0	25.1	27.5	26.5
60	29.1	29.4	32.1	30.7
70	33.1	33.4	36.3	35.1
80	36.7	37.1	40.6	39.1
90	40.3	40.6	44.5	43.1
100	43.6	43.8	48.0	46.2

In addition it seems to me that the Sellier distillation apparatus should not be used for wines containing such low volatile acid content. The variations achieved are too great even in duplicate analyses.

T. E. Twinning.—Variations in results are mostly due to bringing sample to boiling before test is made. Even a few seconds of boiling will account for an appreciable loss of acetic acid on a dilute acetic acid solution. If alcohol is present, this loss will be smaller. Carbon dioxide may best be disposed of by subjecting the wine to vacuum for few minutes.

TABLE 6.—*Effect of pH on rate of distillation of acetic acid*

VOLUME DISTILLED	pH			
	2.15	2.73	3.65	4.40
	PER CENT OF ACID PRESENT DISTILLED OVER			
cc.				
10	5.8	5.8	5.5	4.0
20	11.3	11.3	10.6	7.3
30	16.5	16.8	15.6	11.2
40	21.6	21.8	20.1	14.4
50	26.2	26.1	24.5	17.5
60	30.7	30.4	28.6	20.7
70	34.8	34.5	32.5	23.6
80	38.7	38.4	36.1	26.5
90	42.4	42.1	39.7	29.0
100	45.7	45.3	42.8	31.7

J. L. Weinberg.—In the course of the determinations by A.O.A.C. Method II and its modifications, the writer experienced difficulty due to foaming during the distillations of the samples. Substitution of a Kjeldahl-Clark connecting bulb for the Kjeldahl bulb solved this problem.

A preliminary investigation of the effect of several factors on the rate of distillation of acetic acid from solution was made. Acetic acid solutions, containing approximately 0.08 gram of acetic acid per 100 cc., were steam distilled at a constant and reproducible rate, the solutions in the distilling flask being maintained at a constant volume of 100 cc. The results (Table 4–6) indicate that although alcohol reduced the initial rate of distillation of acetic acid, it had but little effect on rate of distillation of volatile acid, but that the pH had a very noticeable effect, the rate of distillation decreasing markedly with increase in pH.

A complete investigation of the four points stressed in the previous report was not made, but some interesting results were obtained in connection with a modified Peynaud[1] procedure. Peynaud had suggested the use of barium hydroxide to remove sulfurous acid from wines. His procedure is essentially as follows:

Neutralize 25 cc. of wine from which the CO_2 has been removed by vacuumization to phenolphthalein by a saturated solution of $Ba(OH)_2$, store 15 minutes, and keep pink by additions every 5 minutes of more $Ba(OH)_2$. Make up to 50 cc., filter rapidly a 40 cc. filtrate corresponding to 20 cc. of wine, add 15 cc. of a solution of tartaric acid 30 grams/liter, distil, and recover 50 cc. of distillate, which corresponds to 80 per cent of the volatile acids in the sample of wine.

To make this method more amenable to the existing conditions it was altered as follows:

Pipet 50 cc. of wine from which the CO_2 has been removed by vacuumization into a 100 cc. volumetric flask and neutralize to phenolphthalein (if white) or to

[1] *Ann. fals.*, **30**, 1–7 (1937).

natural pigment (if red) by a saturated solution of $Ba(OH)_2$. Allow the mixture to stand for 15 minutes and maintain at the phenolphthalein end point by the addition of more $Ba(OH)_2$ if necessary. Then make up to 100 cc. and filter. Transfer 20 cc. of filtrate into the inner Sellier tube of a Hortvet type apparatus, add 1 cc. of H_2SO_4 (1+3) and distil over 100 cc.

This procedure accomplishes two purposes: It removes the sulfur dioxide and it maintains the solution to be distilled at a distinctly acid point, a pH of about 1.0. Some results obtained with this method are given in Table 7. The distillations were made in a modified Fessler still, and no particular attention was given to rates of distillation.

TABLE 7.—*Comparative determinations of volatile acidity before and after defecation with barium hydroxide*

SAMPLE	DESCRIPTION	DIRECT DISTILLATION				MODIFIED PEYNAUD PROCEDURE			
		cc. 0.1045 N BASE			ACIDITY	cc. 0.1045 N BASE			ACIDITY
A	Acetic acid	1.40	1.42	1.36	0.088	1.25	1.25	1.25	0.078
B	Synthetic wine	1.33	1.32	1.35	0.084	1.25	1.15	1.20	0.076
C	Dry white	1.37	1.23	1.29	0.084	1.50	1.45	—	0.094
D	Dry red	6.08	5.32*	6.0	0.380	5.30	5.27	—	0.330
E	Port	1.30	1.28	1.17	0.082	1.55	1.53	1.54	0.096
F	Sherry	1.17	1.12	1.16	0.072	1.34	1.32	1.20	0.084
G	Sauterne type	1.30	1.44*	1.33	0.085	0.70	0.73	0.70	0.044
H	Sauterne type	1.43	1.45	1.50	0.092	0.68	0.67	0.65	0.042
I	Sauterne type	1.98	2.30*	1.92	0.123	0.71	0.67	0.65	0.042
J	Sauterne type	1.17	1.32	1.27	0.079	0.83	0.80	0.77	0.050
K	Dry white	2.00	2.13	2.07	0.130	1.75	1.85	1.80	0.113
L	Dry red	1.58	1.52	1.50	0.096	1.48	1.42	1.50	0.092

* Values not averaged in calculating acidity.

Under these conditions an occasional erratic result is to be expected. It was found that more readily reproducible results were obtained with the modified Peynaud procedure. It is significant that in the sulfited wines, G to K, considerably lower results were obtained by this procedure, while appreciably higher results were found with fortified wines. The analysis of these results, both in regard to recovery of volatile acids and increase in volatility of fixed acids such as lactic, remains to be done.

It is recommended[1] that the distillation procedures be studied further with a view to eliminating chance errors, and that the modification given, or some other modification, of the Peynaud procedure be tested further. ————————

REPORT ON SULFUR DIOXIDE IN BEERS AND WINES

By L. V. TAYLOR, JR.* (American Can Co., Maywood, Ill.),
Associate Referee

Last year, following a study of sulfur dioxide methods for alcoholic and carbonated beverages (Taylor, Beardsley, and Lueck, *This Journal*,

20, 610 (1937)), the Associate Referee recommended that studies on these methods be continued and that collaborative work be conducted.

Accordingly a series of 12 ounce samples of beer and ale was prepared for collaborative studies. Four codes of beer and four codes of ale, each code consisting of two samples, were submitted to collaborators who had expressed a willingness to cooperate in the project. The accompanying directions suggested that one sample of each code be analyzed volumetrically and gravimetrically by the Monier-Williams method and that its duplicate be analyzed by the tentative A.O.A.C. method for beer.

The analysis of each code at the time the samples were prepared indicated that all ale and beer codes represented in the series contained sulfur dioxide in the order of 20 and 30 p.p.m., respectively, as determined by the Monier-Williams method. Prior to submitting the samples to the collaborators, and approximately one month after their preparation, representative samples of each code were again analyzed. These results indicated that no apparent change had occurred in the sulfur dioxide content during the storage period and also that the samples would be suitable for use in comparing the two methods.

Of those receiving samples, only three reported on the work, namely, H. W. Edwards, Department of Agriculture, Lansing, Mich.; J. B. Thompson, North Dakota Regulatory Department, Bismarck, N. D.; and R. A. Osborn, U. S. Food and Drug Administration, Washington, D. C. Analyses were also made at the American Can Company Laboratories by C. L. Beardsley and E. D. Sallee.

The results reported by the collaborators range from 5 to 33 p.p.m. of sulfur dioxide in the beer samples and from 5 to 21 p.p.m. in the ale samples. Because of these wide variations it is impossible to evaluate the results with respect to the procedures, but they indicate that the wide discrepancy might have been due to the chemical changes occurring in the samples before they were analyzed. Following receipt of the collaborative results, the Associate Referee analyzed additional samples of each lot, and these results further indicated that changes had occurred in the sulfur dioxide content of both products.

Although a comparison of the two methods is not possible from the collaborative results, the Associate Referee considers that the suggestions and comments of the collaborators will be of value in future work on the subject and in preparing collaborative samples of products containing sulfur dioxide. In general, the collaborators experienced difficulty from foaming of the beer when using the tentative A.O.A.C. method. Trouble was experienced with blanks in the Monier-Williams method, and in addition the rapid decomposition of the hydrogen peroxide reagent was also pointed out.

The Associate Referee recommends[1] that the collaborative study of sulfur dioxide in beer and ale be continued and that sulfur dioxide in

wine also be studied. Preparation of samples for collaborative analyses appears to present a special problem.

REPORT ON VOLATILE ACIDS IN DISTILLED SPIRITS

By G. F. BEYER (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.), *Associate Referee*

When official Method I[1] for the determination of volatile acids in wine was applied to whiskey, the results were found to be a trifle high when compared with those obtained by official Method II and the evaporation method. When Method I was used in connection with a solution containing 72 grams of acetic acid per 100 liters of 50 per cent alcohol, the correct result was obtained on the distillate, but the residue required 0.3 ml. of 0.05 *N* sodium hydroxide for neutralization. When Method II was applied to the same solution, the result obtained was 1.5 grams low. The Sellier tube used was 8.5 inches long and 1.0 inch in diameter.

Volatile acids in whiskey were also determined by evaporating just to dryness, then adding 10–15 ml. of water, and again evaporating to dryness. The residue was dissolved in about 25 or 30 ml. of neutral alcohol (50 per cent) and diluted to any convenient volume, and the fixed acids were titrated with 0.1 *N* sodium hydroxide. The difference between this figure and that obtained for the total acids represents the volatile acids.

A number of determinations showed that this method of determining volatile acids in distilled spirits compared very favorably with Method II, and that it is likely to give nearer the correct result than either of the other two methods.

RECOMMENDATIONS[2]

It is recommended that the evaporation method and A.O.A.C. Method II be further studied with the view to adopting the former or both as official for distilled spirits. It is further recommended that the cause of the slight loss resulting from the use of Method II be investigated.

REPORT ON ALDEHYDES IN WHISKEY AND OTHER POTABLE SPIRITS

By PETER VALAER (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.), *Associate Referee*

For some time there has been a demand for an improved A.O.A.C. method for the determination of aldehydes in distilled spirits by those who are opposed to colorimetric methods if there is a gravimetric or

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[1] *Methods of Analysis, A.O.A.C.,* 1935, 166.
[2] For report of Subcommittee D and action by the Association, see *This Journal,* 22, 65 (1939).

volumetric method available. Some analysts find it difficult to produce uniform results by the present A.O.A.C. method (sulfite-fuchsin), principally on account of the instability of the sensitive reagents required. Others have found that there is too wide a variation in the aldehyde content of spirits. For example, when the same barrel of whiskey is analyzed at three or six months' intervals, the difference in results seems to be due to errors in analysis, rather than to changes that actually occur in the barrel during aging.

It was decided to select a method based on the original Ripper principle,[1] which had given good results in the Associate Referee's laboratory.

For collaboration purposes there were prepared three large quantities of whiskey, rum, and brandy containing different amounts of aldehydes. Sealed one-half pint samples and the method of analysis to be used were sent or given to sixteen collaborators. Their results are shown in the table.

The method was published in *This Journal*, **22,** 73 (1939).

DISCUSSION OF RESULTS

The procedure selected is based on sound chemical principles; it is relatively easy and speedy of manipulation and is also one that has given some measure of satisfaction to the users.

In reply to a request for suggestions for improving the method submitted several collaborators offered some interesting and helpful ones. No one was positively opposed to the method; and only one collaborator preferred the present method because he was familiar with it and had designed special equipment for it. It may be well, therefore, to retain the present method as an alternative method.

Several collaborators stated that they would prefer this proposed method to the present A.O.A.C. method provided it produced satisfactory results. It was believed by others that the method would give better results than those submitted when the analysts had had more experience in using it. One chemist stated that it is entirely satisfactory from the standpoint of laboratory operation, but that the reason for standardizing the iodine and bisulfite solutions was not apparent. If exactly 25 cc. of bisulfite solution and 30 cc. of iodine solution are added to each sample and to several controls, the aldehyde is equivalent to the difference in the volume of the thiosulfate solution required for the sample and for the controls. He makes reference to the work of Joslyn and Comar.[2]

Another chemist makes the following most serious objection:

The sodium bisulfite method shows more aldehydes present than there actually are, and the magnitude of the error increases as the aldehyde concentration diminishes. The error becomes very high in the range of aldehyde concentrations we normally find present in whiskey.

[1] *Monatsh.*, **21,** 1079 (1900).
[2] *Ind. Eng. Chem. Anal. Ed.*, **10,** 364 (1938).

Still another chemist writes as follows:

We feel that the fuchsin-sulfurous acid method for aldehydes is quite satisfactory for rapid determinations. However, its limit of error is very great, due to the small amounts of distillate used in the determination. This makes it almost useless for research work of any comparative nature. Thus, you can readily understand that it was with a great deal of interest that the present collaborative work was followed.

It would be very difficult at the present moment to state definitely the cause for the above description. Possibly the work of other collaborators in conjunction with our own will give us a clue.

There are several suggestions which could be used to render the proposed test more accurate. The distillate receiver should be kept in an ice bath to prevent the volatilization of some of the aldehydes during the distillation process. Thus the condensation of aldehydes from the distillate would be independent of the atmospheric conditions of the room.

Collaborative results on aldehydes in whiskey and other potable spirits
(grams per 100 liters)

COLLABORATOR	WHISKEY	RUM	BRANDY
G. F. Beyer	3.85	8.60	43.6
	3.40	8.25	44.6
		8.80	45.1
Loren Burritt	3.00	7.26	43.12
	3.08	7.04	40.40
	3.08		40.00
Peter Valaer	3.30	7.70	40.30
	3.74	7.40	42.90
	3.74	7.90	42.90
C. L. Tucker	3.36	8.40	47.10
		8.10	46.00
A. C. Garland	3.04	8.32	44.00
—. Edwards	3.52	8.58	46.86
	4.40	9.02	47.52
—. Whiting	3.96	9.90	49.94
	3.96	9.68	
Geo. Hamill	3.41	8.40	42.4
	3.28	8.07	42.1
	3.39	8.73	44.2
C. T. Carson	5.52	10.93*	44.97
M. C. Brockman	2.71	8.47	45.60
	2.86	9.28	48.80
Bertha Schwartz	3.41	8.23	32.4*
A. Herman	2.64	8.82	46.52
	2.66	8.77	45.27
Bernard Robb	4.02	8.80	42.03
	3.74	8.36	40.55
John B. Wilson	3.10	8.40	44.1
	3.20	8.40	44.7
Averages	3.46	8.43	44.2

* Averages do not include the lowest brandy or highest rum.

A further standardization of the method could include the designation of the temperature of the sample at the time of pipetting. If the temperature is kept at about 20° C. or lower there will be no loss of the lower-boiling point constituents in the pipetting.

While all the comments could not be included without making this report unduly voluminous, the general trend of opinion may be deduced from the foregoing discussion.

It is recommended[1] that this work be continued for another year in order that improvements based upon the suggestions and experiences obtained may be made.

REPORT ON DETECTION OF ADULTERATION OF DISTILLED SPIRITS

By S. T. SCHICKTANZ (Alcohol Tax Unit, Bureau of Internal Revenue, Washington, D. C.), *Associate Referee*

No definite report will be given at this time, but the Associate Referee should like to discuss briefly the problems encountered during the year.

One of these problems relates to the potentiometric titration of the acids in alcoholic beverages. Two values that were obtained by this method are significant. They are the initial *p*H and the shape or contour of the titration curve, both of which are dependent to a large degree on two factors, temperature and dielectric constant. The *p*H usually increases with a decrease in dielectric constant; or, in other words, the *p*H increases with an increase in concentration of alcohol. To make the values obtained significant and relative, it is necessary to make the titration at an agreed temperature and at a definite dielectric constant. However, the same results may be obtained by making determinations at any temperature and any concentration of ethyl alcohol, and correcting back to a standard basis by means of correction charts. Work has been done on these correction charts during the past year, but due to errors, presumably experimental, in the results of various collaborators, it seemed advisable to obtain more data before presenting these tables.

Another interesting procedure is the chromatographic method of analysis. It has been shown by several German investigators that it is possible to identify coal tar dyes used in the coloration of wines. Much more work must be done, however, to make this method applicable to alcoholic beverages in general. In conjunction with this problem, it is necessary to use such methods as spectrophotometric and ultraviolet absorption.

A new method for the determination of fusel oils is also being investigated. All that can be said for this method at the present time is that it requires approximately three hours for an analysis. The results are in-

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 65 (1939).

dependent of the presence of esters and aldehydes, and the yields are better than 83 per cent of the original fusel oils present.

It is suggested that work on these problems be continued and that results and recommendations be submitted to the committee for approval in the near future.

The report of the Associate Referee on Wood Alcohol in Brandy is included in his paper, entitled "Application of the Neutral Wedge Photometer to the Quantitative Determination of Methanol in Distilled Spirits," published in *This Journal*, 22, 151 (1939).

REPORT ON CORDIALS AND LIQUEURS

By JOHN B. WILSON (U. S. Food and Drug Administration,
Washington, D. C.), *Associate Referee*

An apricot cordial and a peach cordial were prepared by the Associate Referee for analysis as a means of combining the collaborative study of the determinations of esters, benzaldehyde, and gamma-undecalactone.

In each case 1 kg. of the dried fruit was ground in a food chopper, placed in a 1 gallon bottle, and macerated with 2 liters of dilute alcohol (1+1) for 4 days, during which period the contents of the bottles were shaken vigorously several times a day. The liquid portions were then strained through cheese-cloth and reserved in large bottles. Second and third extracts were made in a similar manner, and the maceration was continued for a week. The three extracts of each fruit were mixed and made up to about 7 liters with water.

Determinations of volatile acids and esters were made upon the steam distillates from 300 cc. of each of these mixtures. After these determinations had been made the solutions were rendered alkaline and evaporated as described under the A.O.A.C. tentative qualitative test for gamma-undecalactone, and the lactones were separated. Before the test was made the lactone residue was dried over sulfuric acid and weighed. The odor of the residue was noted and the qualitative test made. The results are given in Table 1.

TABLE 1.—*Analysis of extracts of dried apricots and peaches, with alcohol (1+1)*

EXTRACT	APRICOT	PEACH
Quantity distilled (cc.)	300	300
Dried fruit represented (grams)	43	43
Volatile acid (acetic) per liter (gram)	0.26	0.12
Volatile esters (ethyl acetate) per liter (gram)	0.009	0.012
Residue, lactone separation per liter (gram)	0.015	0.015
Gamma-undecalactone crystals	None	None
Gamma-undecalactone odor	None	None

The next step in making the cordials was that of preparing an artificial flavor that would contribute the correct proportions of esters and gamma-undecalactone. A quantity of oil of cognac was obtained, and its ester content was determined in terms of ethyl acetate. It was found to contain no gamma-undecalactone. The saponification value of a commercial preparation labeled "Peach Aldehyde" was also determined in terms of ethyl acetate, and gamma-undecalactone was shown to be present both by the odor of the residue from the extraction for lactones and by a qualitative test.

About 10 grams of the gamma-undecalactone (peach aldehyde) and 21 grams of oil of cognac were then dissolved in 95 per cent alcohol and diluted to 2 liters. A portion (25 cc.) of the solution was added to 225 cc. of water and steam distilled. The distillate, when neutralized and saponified, was found to contain 132 mg. of esters as ethyl acetate, equivalent to 5.36 grams of ethyl acetate per liter of solution.

The two cordials were then prepared from the fruit extracts and synthetic solution, with the addition of sugar sirup, water, alcohol, and oil of bitter almonds in the proportions given in Table 2.

TABLE 2.—*Ingredients used in making apricot and peach cordials*

	APRICOT	PEACH
Fruit extract (liters)	7.0	6.7
Sirup containing 3.2 kg. sucrose (liters)	4.0	4.0
Artificial flavor solution (liter)	0 8	1.0
Alcohol 95% by volume (liters)	2.2	2.3
Oil of bitter almonds (grams)	2.08	1.25
Total volume (liters)	14.0	14.0

About 1 liter of each cordial was submitted to eight collaborators with instructions to make the determinations listed below.

1. *Alcohol by volume:* Use Method II, p. 170, 5.[1]

2. *Total Solids:* Use 37(c), p. 180.[1]

3. *Benzaldehyde:* Use 100 cc. of sample, following 56, p. 182, except to use two 10 cc. portions of alcohol 30% by volume for the final washing of the precipitate instead of 10% alcohol.

4. *Esters as ethyl acetate:* Determine in duplicate on 300 cc. of sample using 46, p. 181.[1]

5. *Gamma-undecalactone:* Unite the duplicate solutions from the determination of esters and treat as directed in 47, p. 181. Use a tared beaker for the evaporation of the final ether solution containing gamma-undecalactone, dry in a desiccator over H_2SO_4, and report the weight of the residue. If your laboratory is not equipped for the microscopic examination of the crystals of hydrazino-gamma-undecalactone, transfer them to a small vial and mail to the Associate Referee, who will arrange for their examination.

The reports of the collaborators are incorporated in Table 3.

[1] *Methods of Analysis, A.O.A.C.*, 1935.

TABLE 3.—*Analysis of apricot and peach cordials*

COLLABORATOR	BATTISTA	BURRITT	CHRISTENSEN	EDWARDS	SMITH	VALAER	WHITING	WILSON	PRESENT
Determination									
Apricot Cordial									
Alcohol by volume (%)	41.30 / 41.28								
Solids per 100 cc. (grams)		37.66	36.76	36.84	35.99	37.66	37.12	37.56	
Benzaldehyde per 100 cc. (mg.)		24.91	26.44	24.7[1]	23.04[2]	24.91	24.6[1]	23.78	
Esters as ethyl acetate per 100 cc. (mg.)		203	18.1	14.1	13.7	181	13.3	15.8	14.8
Esters as ethyl acetate per 100 cc. (mg.)	24.2	28.2	32.3	26.0	26.4 / 28.9	26.7	27.4	26.4	31.1
Gamma-undecalactone per liter (mg.)	6.0	30.8	23.7	14.6	44.0	33.5	7.0	249.3	357
Qualitative	+	+	–	+	+	+		+	
Peach Cordial									
Alcohol by volume (%)	41.44 / 41.46	39.50	37.76	38.56	38.14	39.95	38.60	39.24	
Solids per 100 cc. (grams)		24.8	27.37	24.3[1]	22.30[2]	24.8	24.4[1]	23.55 / 23.19	
Benzaldehyde per 100 cc. (mg.)		159.0	9.2	11.0	9.1	132.0	7.1	10.0	8.9
Esters as ethyl acetate per 100 cc. (mg.)	26.4 / 26.56	33.7	56.0 / 38.4	34.5	lost	35	35.9	35.2 / 35.5	38.8
Gamma-undecalactone per liter (grams)	6.1	37.4	36.6	17.3	lost	38.5	13.8	185.7	286
Qualitative	+	+	+	+		+		+	

[1] Determinations were made by diluting the residue from the alcohol determination to 50 cc. and taking the immersion refractometer reading.
[2] Calculated from the sp. gr. of the dealcoholized sample.

Additional results on solids were submitted by some of the collaborators as given in Table 4.

TABLE 4.—*Additional results on solids in cordials*

METHOD USED	APRICOT CORDIAL	PEACH CORDIAL
	g./100 cc.	*g./100 cc.*
Wilson: Dried in vacuo on dry asbestos 18 hrs. at 70° C.		
Sample 3 cc.	25.02	24.79
Sample 5 cc.	24.94	24.53
Burritt: Dried in air oven at 100° C. to constant weight	25.07	25.52
Valaer:	25.37	25.95
Smith: Calculated by the formula Sp. gr. sample −sp. gr. distillate / 0.00386	22.84	22.11
From sp. gr. of dealcoholized sample	23.04	22.30

Dilute alcohol, 30 per cent by volume, was chosen to wash the precipitated benzaldehyde phenylhydrazone because it had been noted that in preliminary experiments somewhat high results were obtained when the precipitate was washed with 10 per cent alcohol, as directed in *Methods of Analysis, A.O.A.C.* In these cases a strong odor of esters and gamma-undecalactone was noted, but when the drying was continued for 24 hours or more the precipitate gradually approached the proper amount and the odor was less noticeable. When pure benzaldehyde phenylhydrazone was dried at 70°C. in a vacuum, practically no loss of weight was found after 24 hours.

Another experiment indicates the possibility of an increased percentage of alcohol as a precipitation medium to prevent inclusion of flavoring ingredients by the precipitated phenylhydrazone. A stock solution of benzaldehyde was prepared, and precipitations were made with the phenylhydrazine reagent in several strengths of alcohol. The data are given in Table 5.

TABLE 5.—*Precipitation of benzaldehyde phenylhydrazone in various strengths of alcohol*

ALCOHOL	BENZALDEHYDE PHENYLHYDRAZONE	BENZALDEHYDE
per cent	*gram*	*gram*
10	0.0860	0.0465
20	0.0858	0.0464
30	0.0837	0.0453
40	0.0824	0.0446

A further experiment was performed with the same stock solution in which the benzaldehyde phenylhydrazone was precipitated as usual in a

medium of 10 per cent alcohol and the precipitate washed with other strengths of alcohol.

TABLE 6.—*Recovery of benzaldehyde phenylhydrazone when washed with stronger alcohol*

ALCOHOL FOR WASHING	BENZALDEHYDE PHENYLHYDRAZONE	BENZALDEHYDE
per cent	*gram*	*gram*
30	0.0852	0.0461
	0.0861	0.0466
40	0.0836	0.0452
	0.0800	0.0433

These experiments indicate that the precipitate of benzaldehyde phenylhydrazone may be washed with alcohol, 30 per cent by volume, with comparative safety but that stronger alcohol should not be used. Further work needs to be done to establish whether or not alcohol as strong as 30 per cent by volume may be used as a medium for precipitation of benzaldehyde phenylhydrazone without material loss.

DISCUSSION OF RESULTS

No estimate of the alcohol or solids content of the samples could be made owing to the method of manufacture. A fair agreement was attained by the various collaborators on these two determinations, but better agreement would be desirable.

Except for those of two collaborators, the results for benzaldehyde are remarkably good, the average for the apricot cordial being 15 mg. per 100 cc. as compared with 14.8 mg. calculated. The average for the peach cordial is slightly high, 11.6 mg. per 100 cc., against the calculated 8.9 mg. per 100 cc. It is hoped that slight modifications in the procedure will result in even better results next year.

While fair agreement is shown among collaborators for the ester content of the two samples, there is a discrepancy between the esters found and calculated. It appears to be due to the fact that the esters used were not completely soluble. A thin film of oily matter was found upon the remaining stock of cordials some time after the samples had been distributed. This accounts for the somewhat low results in a number of cases.

The majority of the collaborators succeeded in extracting the gamma-undecalactone from both samples, but the weight of material obtained does not show that this procedure can be used as a quantitative method in its present form. However, it is hoped that some modifications that have already been subject to trial will enable the Associate Referee to develop a quantitative procedure for this substance.

It is recommended[1] that further collaborative work be done on the determination of benzaldehyde, volatile esters, and gamma-undecalactone in cordials.

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 65 (1939).

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MacIntire (University of Tennessee Agricultural
Experiment Station, Knoxville, Tenn.), *Referee*

The 1937 report of the Referee carried five recommendations for continued work, and a recommendation that a study of methods for the determination of fluorine in soils be initiated. All of these recommendations have been in operation during the past year. The Referee has served only in an advisory capacity for the work that was already under way at the 1937 meeting. An imperative need had arisen, however, for a dependable technic for the recovery of fluorides from soils of known experimental history and for the determination of the fluorine content of crops grown thereon. The Referee and his associate, J. W. Hammond, therefore, pursued a study of the operations that would bring all forms of fluorides into solution for distillation by the Willard-Winter procedure. The resultant technic was transmitted to the Associate Referee, whose findings and conclusions will be embodied in his own report.

The method that was developed from the determination carried out by Hammond is here given, with tables of experimental data and observations relating thereto.

USE OF PEROXIDES OF CALCIUM AND MAGNESIUM IN DETERMINATION OF FLUORINE CONTENT OF SOILS, SILICEOUS MATERIALS, AND ORGANICS*

By W. H. MacIntire and J. W. Hammond

The following directions were submitted to the collaborators:

I. SOILS

Sampling.— Follow the procedure given in *Methods of Analysis, A.O.A.C.,* 1935, 1, and then grind 10 grams of the air-dried soil to 325-mesh in an agate mortar; mix thoroughly and preserve in a suitable stoppered container.

Distillation Apparatus.—Use either that prescribed by Willard and Winter, *This Journal,* 16, 105 (1933), or the multiple-unit setup recommended by Reynolds, Kershaw, and Jacob, *Ibid.,* 19, 156 (1936).

Determination.—For soils of high fluorine content, use a 0.5 gram charge; for those low in fluorine, use 1 gram. Mix the charge intimately with three times its weight of precipitated CaO_2 in either a nickel or platinum crucible; char thoroughly and then heat at approximately 900° C. for 30 minutes. Cool, and carefully brush the incinerated contents of the crucible into the distillation flask. Wash the walls of the flask with 5 cc. of water; add 3 drops of phenolphthalein; neutralize with 60% perchloric acid and then add 15 cc. additional. Bring to 135° ± 5° C. and maintain the distillation temperature and volume during the collection of a distillate of 200–250 cc. while passing a balanced current of steam through the suspension.

For soils of low fluorine content, concentrate the entire distillate by evaporation prior to titration; for those of high fluorine content, titrate an aliquot of 5–50

cc. Titrate the concentrate or the aliquot with 0.01 N thorium nitrate in a 50% ethyl alcohol solution, using 2 drops of a 0.05% aqueous solution of sodium alizarin sulfonate as indicator.

II. ORGANICS AND NON-SILICEOUS MATERIALS

Sampling.—From a thoroughly mixed air-dried sample take an appropriate sub-sample, as governed by the supposed content of fluorine.

Preparation of subsample.—Grind to less than 1 sq. cm. Mix well and either heat overnight in an oven at 105° C., or dry over H_2SO_4 under reduced pressure for 24 hours, and preserve in a reagent bottle.

Distillation apparatus.—Use that prescribed for "Soils."

Procedure.—Weigh a charge to furnish approximately 1 mg. of fluorine. (This may require as much as 25 grams.) To a 10 gram charge or less, add one-half its weight of MgO_2 and mix in a 200 cc. evaporating dish of either nickel or platinum. When a larger charge is used, add 5 grams of MgO_2 in aqueous suspension; dry the wetted mixture at 105° C. for 24 hours; char slowly; and then incinerate in either an electric furnace or an incinerator at 500°–600° C. (Incineration is complete in 4–6 hours; in some instances, however, 12 hours may be required.)

Transfer the ash to the distillation flask. Neutralize with $HClO_4$ and add an excess of 15 cc. Bring to a temperature of 135° ±5° C. and collect 200–250 cc. of distillate while maintaining that temperature during the passage of a balanced current of steam. Evaporate the distillate to a volume of 10–20 cc. and dilute with an equal volume of alcohol; add 3 drops of 0.05% sodium alizarin sulfonate; adjust reaction with NaOH and HCl, and then titrate with 0.01 N $Th(NO_3)_4$; or follow the zirconium-alizarin colorimetric procedure prescribed by Winter, *This Journal*, 19, 362 (1936).

EXPERIMENTAL

The removal of fluorine from the perchloric acid digestions by means of a balanced current of steam, as used by MacIntire, Shaw, and Hardin[1] in the dissolving of phosphates, was compared with removal from boiling

TABLE 1.—*Effect of a balanced current of steam upon the recovery of fluorine by distillation*

MATERIAL	CHARGE	FLUORINE RECOVERY FROM DISTILLATION VOLUME MAINTAINED	
		BY ADDITIONS OF WATER	BY CURRENT OF STEAM
	grams	*per cent*	*per cent*
Rock phosphate[a]	0.5	3.58	3.64
W.D. triple phosphate	1.0	1.80	1.85
Tenn. brown rock phosphate	0.5	3.39	3.40
		p.p.m	*p.p.m.*
Soil used in pot experiment No. 2	1.0	110	110

[a] Bureau of Standards sample No. 56.

digestions kept to volume by water replacement. The distillations (Table 1) show that substantially identical results were obtained by the two

[1] *Ind. Eng. Chem.*, 10, 143 (1938).

TABLE 2.—*Effect of variable prior treatment of soils, as influencing recovery of fluorine by steam distillations*

SOIL AND CHARGE	FLUORINE ADDITURE	TREATMENT PRIOR TO DRYING AND INCINERATION	HEAT TREATMENT		FLUORINE RECOVERY	
			TEMPERA-TURE	TIME		
grams	grams		°C.	hrs.	grams	per cent
Red Clay[a] 0.3185	0.00933[b]	None	500	2	0.00873	94.5
0.3478	0.00468[c]	None	500	2	0.00402	88.8
0.3621	0.01222[b]	Wetted with 5 cc. of 5% solution of sucrose	500	2	0.00974	79.8
0.6386	0.01543[b]	Wetted with 2 cc. of 5% solution of sodium acetate	500	2	0.01542	99.9
0.8250	0.0065[b]	Wetted with 0.75 gram of $Mg(NO_3)_2$ in solution	500	2	0.0023	34.0
0.8241	0.0065[c]	Mixed with 2 grams of MgO_3	500	2	0.00278	42.5
1.0000	0.01894[b]	Mixed with 3 grams of CaO_2	900	4	0.01898	100.2
0.5000	0.01762[c]	Mixed with 1.5 grams of CaO_3	900	4	0.01742	99.0
0.5000	0.01450[c]	Mixed with 1.5 grams of CaO_3, wetted, and dried at 105° C.	900	4	0.0145	100.0
Hartsells fine sandy loam[d] 0.4569	0.01750[b]	None	500	2	0.01749	99.8
0.3489	0.00986[b]	Wetted with 5 cc. of 10% suspension of $MgCO_3$	500	2	0.00526	53.4
1.0000	0.01105[c]	Wetted with 0.75 gram of $Mg(NO_3)_2$ in solution	500	2	0.00994	90.0
1.0000	0.01105[c]	Mixed with NH_4Cl and $MgCO_3$[e]	800	2	0.0020	18.1
1.0000	0.01105[c]	Mixed with NH_4Cl and $CaCO_3$[e]	800	2	0.0022	19.9
1.0000	0.01105[c]	Mixed with NH_4Cl and $BaCO_3$[e]	800	2	0.0048	43.4
0.5000	0.0211[c]	Mixed with 0.5 gram CaO_2	900	4	0.0211	100.0
1.0000	0.0211[c]	Mixed with 3 grams CaO_2	900	4	0.0211	100.0

[a] Red clay subsoil, laboratory No. 6562, air-dried, and ground to 325-mesh.
[b] Added as CaF_2.
[c] Added as $BaSiF_6$.
[d] Used in T.V.A. pot experiments.
[e] One gram of chloride and 8 grams of carbonate.

procedures. Apparently, however, activation and the "sweeping" of the current of steam decreased by one-half the time requisite for complete removal of fluorine. The passage of steam also eliminates bumping, frequently encountered and especially with highly siliceous materials.

Effect of elevation of temperature was also injected into the comparisons. Variations in the range of 135°–145°C. showed no definite influence upon the effectiveness of the fluorine recoveries.

Influence of variable treatment prior to steam-current distillation

Table 2 shows the recoveries obtained from a red clay and an experimental soil that were fortified by variant additions of calcium fluoride and barium silicofluoride and then subjected to ignitions with different calcic and magnesic compounds. Liberation of fluorine by perchloric acid digestion was greatly repressed by the several magnesic materials—nitrate, carbonate+ammonium chloride, and peroxide—that were used in the ignitions precedent to distillation.

Apparently the generated magnesic oxide induced silicate combinations from which fluorine is not liberated by the perchloric acid. A similar repressive effect was induced by the 8 to 1 mixtures of the carbonates of calcium and barium with ammonium chloride. The opposite effect—complete recovery of the added fluorine—was obtained by the use of calcium peroxide in the ignitions that preceded distillations.

The peroxide ignitions imparted a splendid physical condition to the soil residues and probably induced formation of calcium fluoride from which ready release of fluorine is effected by the perchloric acid digestion.

Calcium peroxide incinerations vs. sodium-potassium carbonate fusions

Eleven experimental soils that had received treatments of different fluorides, over a period of 8 years, were subjected to ignition with calcium peroxide in comparison with sodium-potassium carbonate fusion as treatments precedent to perchloric acid distillation. The fusion procedure was that prescribed in Bureau of Standards Research Paper 110.

The results (Table 3) demonstrate that the calcium peroxide ignitions induced the highest recoveries from the soils that had been treated with fluorides years previously. In some cases fluorine recovery from the fusions was less than that from the soil given no treatment immediately prior to the perchloric acid distillation. Such disparities may be accounted for by the fact that the carbonate fusions developed siliceous colloidal material that repelled evolution of fluorine during the perchloric acid digestion. During such digestion the charges that had been ignited with calcium peroxide maintained a physical state much superior to that which characterized the carbonate-fusion charges during their digestion with perchloric acid.

TABLE 3.—*Calcium peroxide incinerations vs. sodium-potassium carbonate fusions as prior treatments for the perchloric acid distillation of fluorine from soils*

SOIL[a]	ANALYTICAL CHARGE	PRIOR CARBONATE FUSION[b] OR PEROXIDE INCINERATION[c]	FLUORINE RECOVERY[d]
Pot experiment	*grams*		*p.p.m.*
	0.5	None	169
No. 1	2.0	K_2-Na_2CO_3	100
	1.0	CaO_2	205
	0.5	None	96
No. 2	2.0	K_2-Na_2CO_3	98
	1.0	CaO_2	185
	0.5	None	80
No. 3	2.0	K_2-Na_2CO_3	177
	1.0	CaO_2	315
	0.5	None	100
No. 4	2.0	K_2-Na_2CO_3	117
	1.0	CaO_2	154
	0.5	None	65
No. 5	2.0	K_2-Na_2CO_3	95
	1.0	CaO_2	138
	0.5	None	108
No. 6	2.0	K_2-Na_2CO_3	281
	1.0	CaO_2	488
Lysimeter			
	0.5	None	84
No. 6569	2.0	K_2-Na_2CO_3	70
	1.0	CaO_2	132
	0.5	None	166
No. 6570	2.0	K_2-Na_2CO_3	338
	1.0	CaO_2	372
	0.5	None	676
No. 6571	2.0	K_2-Na_2CO_3	388
	1.0	CaO_2	743
	0.5	None	187
No. 6572	2.0	K_2-Na_2CO_3	376
	1.0	CaO_2	472
	0.5	None	225
No. 6573	2.0	K_2-Na_2CO_3	209
	1.0	CaO_2	402

[a] Soils were from lysimeters and T.V.A. pot experiments. Last four lysimeter soils had been treated with $BaSiF_6$.
[b] The procedure outlined in Bureau of Standards Research Paper 110.
[c] 0.5 gram of CaO_2 was mixed with 0.5 gram of soil; incineration for 30 minutes at 900°C.
[d] Averages of two to four determinations per sample.

Calcium peroxide vs. magnesium peroxide for organics

The data of Table 2 demonstrate the adaptability of calcium peroxide, and the inadmissibility of magnesium peroxide, as oxidants and for incinerations of soils and siliceous materials. Comparisons were therefore made to determine the comparative values of the several calcic and magnesic materials of Table 4 in the pre-treatment requisite for determination of the fluorine content of organics by perchloric acid distillation.

TABLE 4.—*Comparative recoveries of fluorine from organic material[a] by incineration with basic compounds and distillation with perchloric acid*

| FLUORINE ADMIXTURE[b] | ADMIXED BASIC COMPOUND | | INCINERATION | | FLUORINE RECOVERY |
	COMPOUND	CHARGE	TEMPERATURE	TIME	
grams		*grams*	*°C.*	*hours*	*per cent*
0.00905	$CaCO_3$	1.0	550	12	94.0
0.0181	CaO_2	1.5	Loss due to explosion		
0.0226	$Mg(NO_3)_2$	1.0	500	2	79.0
0.0452	MgO	3.0	550	6	96.0
0.0226	MgO_2 and	2.0			
	$NaC_2H_3O_2$	1.0	600	4	81.5
0.0181	MgO_2	2.0	600	4	100.1
0.0452	MgO_2	3.0	750	1	96.0
0.0226	MgO_2	2.0	600	4	100.0
0.0091	MgO_2	2.0	500[c]	6	100.1

[a] Constant charge of 5 grams of millet.
[b] Fluorine enrichment by admixture of sodium fluoride.
[c] Ashed over a low flame; other incinerations were made in a muffle furnace.

Complete recovery of the fluorine supplied by admixed sodium fluoride was obtained only by the use of magnesium peroxide. Ideal incinerations and complete fluorine recovery were obtained when a mixture of 5 grams of millet and 2 grams of magnesium oxide were incinerated for periods of 4–6 hours in the range of 500°–600° C. The reaction between the peroxide of calcium and the organic matter was, however, so violent that it was deemed inadvisable to try to adjust workable proportions, particularly since the peroxide of magnesium proved such an effective and workable oxidant.

For materials of low organic and high siliceous content, peroxide of calcium is therefore advocated to the exclusion of magnesium peroxide. For materials of high organic and low siliceous content the peroxide of magnesium is preferred.

It is recommended[1] that the proposed procedure be subjected to further collaborative study by the Associate Referee.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 53 (1939).

REPORT ON HYDROGEN-ION CONCENTRATION OF SOILS OF ARID AND SEMI-ARID REGIONS

By W. T. McGEORGE (College of Agriculture, University of Arizona, Tucson, Ariz.), *Associate Referee*

In cooperation with the members of the Western Society of Soil Science a study of several methods of determining pH of arid soils is now underway. It involves different soil-water ratios using both distilled water and tap water. It has been found that when alkali soils are diluted with water for a pH determination the values obtained are much higher than the pH of the soil at field moisture content. However, if tap water, which is fairly well buffered, is used for the dilution, the value obtained more closely approaches that in the field than does the value obtained with distilled water. For this reason the use of tap water is being studied.

While there is nothing to report on this work at present, it is hoped that this data will be ready by the next annual meeting. It is recommended[1] that the variation of type of water used and its ratio to soils be studied further.

No report on hydrogen-ion concentration of soils of the humid regions was given by the associate referee.

REPORT ON LIMING MATERIALS

By W. M. SHAW (University of Tennessee Agricultural Experimental Station, Knoxville, Tenn.), *Associate Referee*

The Associate Referee studied the reactivity of limestone in boiling ammonium chloride solution. Special consideration was given to dolomitic limestones from different regions, since this material is being used extensively to condition and to supply nutrient magnesium in mixed fertilizers. The experimental procedure and some of the results on the reactivities of dolomitic limestones of varying degree of fineness will be followed by a more detailed presentation.

PROCEDURE FOR THE DETERMINATION OF REACTIVITY OF LIMESTONES
CHEMICAL PRINCIPLE

A suspension of calcic or dolomitic limestone in a solution of ammonium chloride will react according to the equations—

1. $CaCO_3 + 2NH_4Cl \rightleftharpoons CaCl_2 + (NH_4)_2CO_3$.
2. $Ca \cdot Mg(CO_3)_2 + 4NH_4Cl \rightleftharpoons CaCl_2 + MgCl_2 + 2(NH_4)_2CO_3$.

When the suspension is heated the $(NH_4)_2CO_3$ is volatilized. The distillate is condensed and trapped in standard acid to afford a measure of the rate of reaction. Calcic and dolomitic limestones show marked differences in reactivity and in the

effect of crystallinity and other physical properties. Reactivity will be affected also by the factors of mass, fineness, concentration of reagent, temperature of the suspension, agitation, and rate of removal of volatile by-products. In a study of any
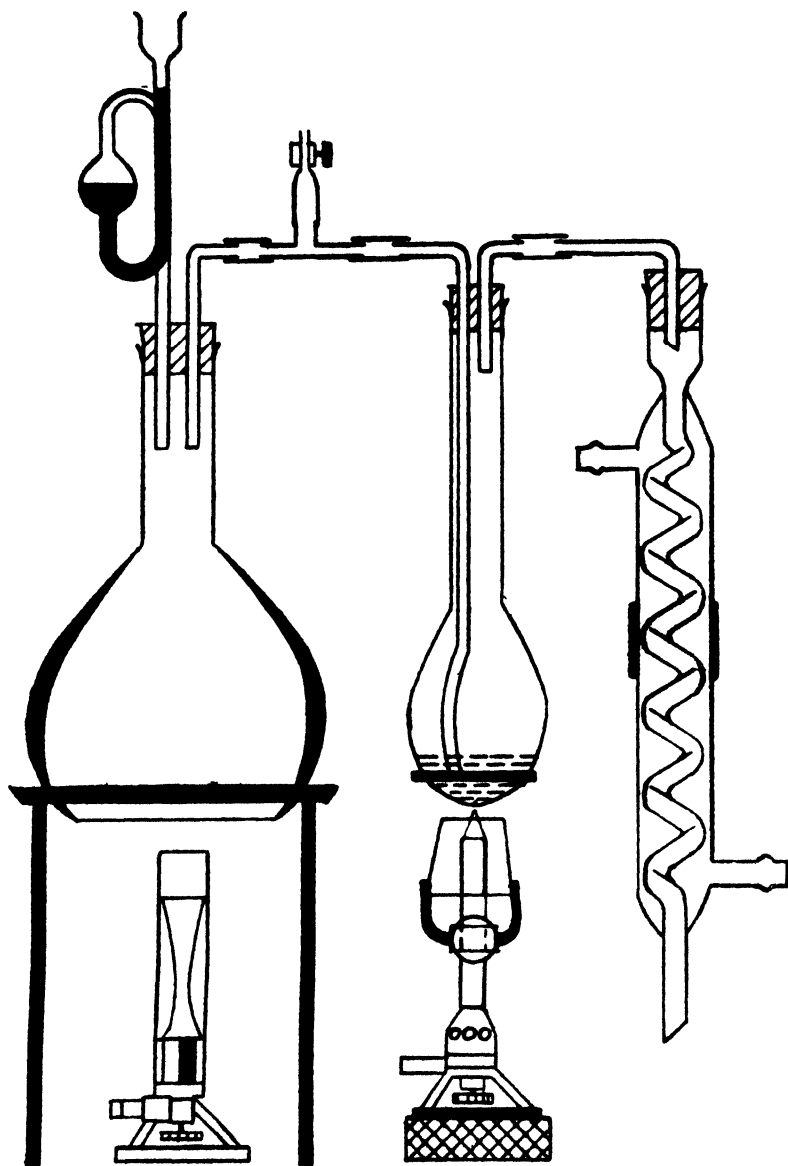
FIG. 1.—AMMONIUM CHLORIDE STEAM DISTILLATION APPARATUS FOR THE EVALUATION OF LIMESTONES

one of those factors, it is essential to maintain all other variables constant. This objective was sought in proposing the apparatus and the procedure herein described.

APPARATUS

(1) The apparatus is shown in Figure 1. The steam generator consists of a 2 liter Pyrex boiling flask, provided with a mercury pressure gage and steam outlet. (2) The reaction is effected in a 500 cc. long-necked Kjeldahl flask provided with a 2 holed rubber stopper carrying 7 mm. glass inlet and outlet tubing; the inlet tube should be so bent as to give a spoon-like outline inside the flask, with an opening constricted to 3 mm. and resting about 7 mm. from bottom of flask slightly off center to the left. (3) The 14 inch condenser is of the spiral type. (4) Graduated beakers, 150 cc. capacity. (5) One Bunsen burner and one Fisher burner. (6) A "T" tube between the steam generator and the digestion flask to allow opening and closing of the steam line.

PROCEDURE

Heat the steam generator to boiling and maintain that temperature. Regulate the heat to vaporize 100 cc. of water every 5.5 minutes. Introduce a 0.25 gram charge of either limestone or dolomite into the reaction flask. Measure the required amount of 0.1 N HCl into a graduated beaker, add 2 drops of 0.1% methyl orange indicator, and place the beaker under the condenser. Deliver into the reaction flask 100 cc. of 2 N NH$_4$Cl(C.P.), washing down the adhering material. Connect the reaction flask with the condenser and steam line and bring the steam generator to vigorous boiling by means of the Fisher burner, simultaneously closing the steam line and applying heat to the reaction flask. Maintain the flame under the reaction flask one-inch high and one inch from the bottom of the flask. Collect successive 100 cc. distillates. Titrate the distillate to a change from golden yellow to orange yellow. This titration times 2 expresses the per cent CaCO$_3$ equivalence of sample for that distillation period.

RELATIVE AVAILABILITY OF LIMESTONE SEPARATES

In applying the above procedure to 100-mesh limestone, complete decomposition was effected during the digestion period requisite for a single distillate of 100 cc. If this value is used as 100 per cent availability, a relative value can be assigned any dolomite.

The experimental results on the availability of different separates of a number of typical dolomites are presented in Table 1. It will be observed that in the 20–40-mesh separate four of the six dolomites show an availability of 15±per cent. The other two are known to be exceptional. The one of low availability was an exceptionally hard rock, and the one of

TABLE 1.—*Availability of dolomitic limestone separates and surface reactivity constants for various dolomites*

SOURCE OF DOLOMITE	AVAILABILITY (PER CENT OF CHARGE)					SURFACE REACTIVITY K
	−20 +40	−40 +60	−60 +80	−80 +100	−100 +140	
Kelley Island Lime Co.	16.0	24.2	32.8	37.4	49.0	25
Ladd Lime Co.	14.0	20.6	26.6	34.4	40.4	20
Warner Co., Sample A	12.6	18.2	21.0	25.2	29.0	14
Warner Co., Sample B	15.6	23.6	30.6	36.4	45.0	23
Standard Lime Co.	14.1	22.6	30.8	36.8	45.0	23

much higher value contained calcium carbonate in excess of the dolomitic ratio. The results of each of the normal dolomites show a fairly constant progression in availability with each decrease in particle size. To establish a more fundamental relationship between particle size and availability, the computed surface area of each dolomite separate was plotted against degree of availability (Figure 2). The figures on the base line show the coincidence between each separate and its computed surface area.
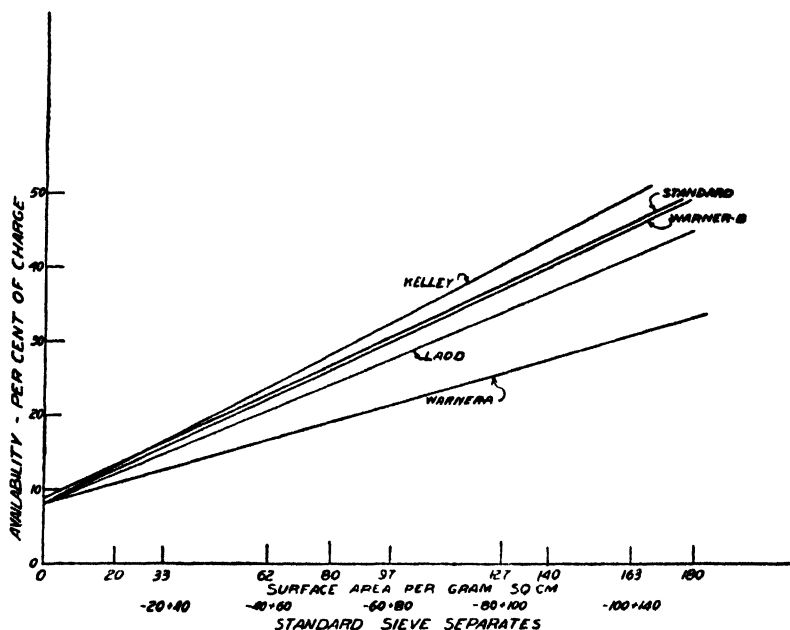
FIG. 2.—AVAILABILITY OF DOLOMITIC LIMESTONES AS AFFECTED BY SURFACE AREA OF SEPARATES

It should be noted that the graphs of Figure 2 were computed on the assumption of spherical particles, although such uniformity in shape does not obtain. Moreover, the mean of diameter taken was the mean of the openings of the respective pairs of sieves, on the assumption of an even distribution of extremes. This assumption, however, may not be justified for all separates. Nevertheless, distinct and close correlation between the variables was found for each dolomite, as shown by the straight lines in Figure 3. These straight lines indicate that for *this experimental range*, at least, the availability by the proposed method is directly proportional to the surface area of the separates. The relationship between surface and availability may be expressed by the straight line equation: $Y = A + BX$, in which Y is the availability in per cent of charge, X is the surface area per gram of dolomitic separate in terms of square centimeters, A, the

point at which the extension of the experimental lines intersects the Y-axis, which in the experimental range has a common value of 8, and B, which is a characteristic constant of each dolomite. This B value of any unknown dolomite may be determined empirically for one separate or preferably for two separates and the resultant Y values substituted in the formula, $Y = 8 + BX$. For simplicity, making K equal 100B, a surface reactivity constant of each dolomite can be expressed as percentage calcium carbonate equivalence of charge per 100 square centimeter surface per gram material. This K value, determined by averaging the several experimental points for each rock of Table 1, is given in the last column of that table. The evaluation of this constant presents a most sensitive method for detecting heterogeneous composition of a given sample. For example, the Mascot Knox dolomite, which contains about 10 per cent calcium carbonate over the dolomitic ratio, gives the following computed K values for the respective experimental points in Table 1: −49, 34, 33, and 26. Such divergence in K values of the individual experimental points establishes the presence of a carbonate more soluble than the dolomite proper, usually either calcite or aragonite.

Taking 23 as the average K value for normal dolomites, the writers recalculated the availability of the several separates by inserting the surface area of the respective separates in place of X, thus $Y = 8 + .23X$. The following values were then obtained: 15.6, 23.3, and 33.8 for the 20 to 40-, 40 to 60- and 60 to 100-mesh separates, respectively. These mean values are in fair agreement with Taylor and Pierre's[1] experimental data obtained by determination of the carbonate decomposition during a single season, which were 12, 21, and 33 for the respective separates. The writer's outdoor experiments[2] with Mascot dolomite and Cumberland silt loam showed for one season a mean carbonate decomposition about 10 per cent above the laboratory figures for the same dolomite. It is recognized that the reaction between a soil and a dolomite will depend on degree of soil acidity and other factors. The above soil experiments are cited merely to indicate the correlation of the present laboratory data with those obtained from one season's soil contact.

The same procedure may be applied to field samples of limestone to express availability in terms of 100-mesh calcic limestone. Such valuation will reflect the factors of fineness, and also physical and chemical characteristics of the rock.

It is recommended[3] that the ammonium chloride steam distillation procedure for the evaluation of limestone availability be studied further in relation to soil-carbonate reactions in pot experiments.

[1] *J. Am. Soc. Agron.*, **27**, 764 (1935).
[2] *Ibid.*, **22**, 272 (1930).
[3] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 53 (1939).

REPORT ON THE AMMONIUM CHLORIDE-DISTILLATION PROCEDURE FOR THE DETERMINATION OF EXCHANGEABLE BASES IN SOILS

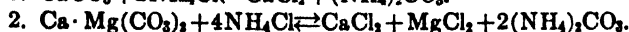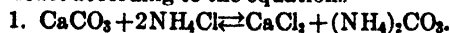By W. M. SHAW (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.)

The work of the Associate Referee on Liming Materials during the past year was directed toward improvements in the technic and accuracy of the ammonium chloride-distillation procedure for the determination of base exchange values of soils. It was thought advisable to postpone collaborative work until the completion of the studies leading to such improvements. The results of those studies follow.

ACCELERATION OF THE AMMONIUM CHLORIDE-SOIL DIGESTION

The procedure of the 1937 report called for digestion of soils with ammonium chloride in 600 cc. covered beakers over a Bunsen burner until a special bromocresol green test paper gave a negative test for ammonium hydroxide in the issuing vapors. Under such circumstances a soil carrying a moderate supply of dolomitic limestone would require 4–5 hours for the complete dissolving of the carbonates. In related studies on the rates of decomposition of limestones and dolomites by ammonium chloride it was found, however, that the passage of steam through the digestion medium greatly expedited the decomposition process. The apparatus (see Figure 1, p. 238) was adopted for soil digestions with ammonium chloride. This assembly consists of a 2 liter boiling flask as a steam generator, a 500 cc. long-necked Kjeldahl digestion flask, and a 14 inch coiled-tube condenser with connections of 7 mm. glass tubing. The inlet tube extends to 7–8 mm. from the bottom of the Kjeldahl flask and is constricted to a 3 mm. opening.

Directions for the present technic are as follows:

Weigh 10 gram charges of soil into the digestion flask, and wash the neck and sides of flask by an addition of 100 cc. of 2 N NH$_4$Cl solution. Connect the digestion flask with the steam generator and with the condenser. Bring the steam generator to boiling temperature and adjust the burner to give a vaporization rate of 100 cc. of water every 5.5 minutes. Place the receiver with 0.1 N acid under the condenser and begin digestion by closing the T-tubes and adjusting the auxiliary burner to give a flame 1 inch high and 1 inch below the bottom of the digestion flask. Collect each successive 100 cc. distillate and determine its titration value until the 0.1 N titer value is reduced to 1.0 cc.

The objective of this procedure was to effect complete disintegration of the carbonates of the soil *without decomposition* of the soil complex. It was therefore essential to determine how readily included calcic and dolomitic limestones are dissolved by the proposed procedure. Accordingly, 9 soils of wide range in texture, organic matter content, and degree of base saturation were selected. Each soil was subjected to the

digestion-distillation process in order to establish its inherent capacity to release ammonia from a boiling solution of ammonium chloride. Each soil was also supplemented with 0.25 gram of high-grade dolomite, and the progressive release of ammonia from these mixtures was compared with such release from the untreated soil. The results are presented in Table 1.

It will be observed that the ammonia released by the unsupplemented surface soils, Series A, shows a wide range, and digestion periods ranging from 11 to 33 minutes against a range of 5.5–22 minutes for subsoils. All the original differences in ammonia release from the surface soils were obliterated in the dolomite-supplemented surface soils and with completion effected by 12 or 13 distillation periods. The dolomite decomposition in the subsoil samples was effected more rapidly, during 9–10 periods. The consistent difference between the order of dolomite decomposition in surface soils and subsoils suggests the possibility that the dolomite is actually decomposed in a shorter time than that indicated in all cases, and that the prolonged release of ammonia from the surface soils is due to a difference between equilibrium between ammonia and organic matter and a corresponding equilibrium between ammonia and the mineral soil complex. It should be noted that the dolomite supplements were much larger than the amounts to be found ordinarily in soils. Moreover, in a soil sample ground to pass a 60 mesh, the dolomite would be much finer than the 60- to 80-mesh fineness taken in these experiments. These considerations point to the conclusion that a 2.5 per cent content of dolomite would be dissolved completely from a soil during a 1 hour digestion by the proposed procedure and that smaller proportions of dolomite would be dissolved in still less time.

The dissolving of calcic limestone by this procedure is so rapid that no problem is presented. This is shown by 3 c. of Table 1. The addition of 2.5 per cent calcic limestone of 60–80 mesh to a highly saturated Dewey silt loam required only one 5.5 minute digestion beyond the single digestion required for the unsupplemented soil.

These experiments established maximal limits of digestion periods for complete dissolving of mineral carbonates under extreme conditions. The actual time requirement for each sample can be established definitely by the ammonia release in successive distillates as above presented. The closed system steam-digestion requires only about one-fourth of the time required for the previously described beaker digestion. Digestion in the Kjeldahl flask has an additional advantage over beaker digestion in that the soil is readily removed from the flask by a jet of the wash solution, whereas both beaker and its cover-glass have to be policed free of adhering soil. This method of digestion also admits of more accurate recognition of the complete dissolving of dolomite present in soils.

TABLE 1.— *Decomposition of 60-80-mesh limestone and dolomite admixed with various soils, as determined by the ammonium chloride-steam distillation procedure*

REF. NO.	SOILS*	ADDITION†	0.1 N TITER OF SUCCESSIVE 100 CC. DISTILLATES													SUM
			1	2	3	4	5	6	7	8	9	10	11	12	13	
1A	Hartsells sandy loam	0	1.5	1.0												2.5
1B	Hartsells sandy loam	Dolomite	14.1	10.8	7.6	5.6	4.5	3.0	2.3	2.0	1.8	1.5	1.3	1.1		
2A	Clarksville silt loam	0	2.3	1.2	.9											4.4
2B	Clarksville silt loam	Dolomite	14.5	10.3	7.5	5.7	4.2	(4.0)	2.5	2.2	1.9	1.6	1.4	1.2	1.0	
3A	Dewey silt loam	0	6.0	2.0	1.5	1.2	1.0									11.7
3B	Dewey silt loam	Dolomite	17.5	10.2	8.3	6.4	5.2	4.2	3.2	2.4	2.0	1.5	1.2	1.0		
4A	Colbert silty clay loam	0	10.3	3.2	2.1	1.6	1.3	1.0								19.5
4B	Colbert silty clay loam No. 6562	Dolomite	20.6	11.7	8.9	7.6	5.7	4.9	3.9	3.1	2.3	1.8	1.3	1.2	1.0	
5A	Decatur silt loam‡	0	35.0	9.3	5.0	3.2	2.8	2.1	1.6	1.3	1.2	1.0				
6A	Cherokee clay subsoil No. 6557	0	.7													.7
6B	Cherokee clay subsoil No. 6557	Dolomite	13.0	12.5	8.7	6.9	4.7	2.8	1.8	1.2	1.0					
7A	Cherokee clay subsoil No. 6561	0	1.9	.7												2.6
7B	Cherokee clay subsoil No. 6561	Dolomite	15.1	12.9	9.0	6.2	4.0	2.4	1.5	1.2	1.0					
8A	Colbert silty clay subsoil	0	6.0	1.6	1.1	.9										9.6
8B	Colbert silty clay subsoil	Dolomite	18.6	12.0	11.4	7.0	5.2	3.6	2.5	1.8	1.3	1.0				
3C	Dewey silt loam	Limestone	42.5	10.4	3.0	1.6	1.2	1.0								

* 10 grams of air-dry soil.
† 0.25 gram of limestone or dolomite.
‡ This soil has a natural dolomite content of 2.19% as $CaCO_3$ equivalence

ACCURACY OF MAGNESIUM DETERMINATION BY THE BOILING AMMONIUM CHLORIDE SOLUTION

In the collaborative study of this procedure in 1937, the magnesium results were discordant and in general higher than those obtained by the Associate Referee. Some of the collaborators suggested that the discordance may be due to decomposition of magnesic minerals by the boiling ammonium chloride solution. To throw some light on this point the Associate Referee made duplicate and triplicate extractions of 8 soils and determined the magnesium content of each extract. The results (Table 2) seem to establish the precision of the magnesium determination. Most of the determinations agree within a few hundredths of 1 mg. equivalent per 100 grams of soils and only 3 of the 20 determinations were out of line as much as 0.2 cc. The higher values obtained by some collaborators in 1937, therefore, cannot be attributed to drastic action of the boiling ammonium chloride solution. The cause for erroneous magnesium values is

TABLE 2.—*Precision and extent of absorbed magnesium determination by extraction with boiling ammonium chloride solution*

SOIL	MAGNESIUM FROM BOILING EXTRACTION			1937 REPORT BY ASSOC. REF.
	1	2	3	
1937 A.O.A.C. No. 1	1.26	1.04	1.16	1.32
1937 A.O.A.C. No. 5	2.08	2.04	—	2.25
1937 A.O.A.C. No. 6	1.04	1.16	1.00	1.10
Cherokee clay subsoil No. 6562	0.66	0 74	—	—
Colbert silty clay loam X-10	2.62	2.66	2.55	—
Colbert silty clay subsoil	1.64	1.60	1.46	—
Becket sandy loam	5.28	5.32	—	—
Becket sandy subsoil	1.18	1.16	—	—

probably incomplete removal of the manganese, which is nearly always present in the extracts. Complete removal of this element can be accomplished by the addition of ammonium persulfate to a slightly ammoniacal solution at boiling heat. The hot ammonium chloride wash solution should be distinctly ammoniacal to preclude the dissolving of manganese during the washing of the hydrated oxide. Contamination of alumina is obviated by the addition of 3–4 cc. of molar citric acid before the precipitation of the magnesium.

EFFECTIVENESS OF ADDED MAGNESIUM OXIDE IN THE DISTILLATION OF ABSORBED AMMONIA IN SOILS

In the determination of absorbed ammonium ions as a measure of exchange capacity the ammonia release and distillation is usually induced by the addition of magnesium oxide to the soil-water suspensions. The released ammonia in successive 100 cc. distillates was determined as a

measure of the speed of ammonia evolution by the magnesium oxide displacement. The ammonia release from soils of high absorptive capacity was prolonged and quite incomplete in distillates of 200 cc. Six soils of high absorption capacity were distilled from aqueous suspensions of limited sodium hydroxide concentration in comparison with distillations in which magnesium oxide was used. The results are given in Table 3.

TABLE 3.—*Comparative efficiency of MgO and NaOH in the distillation of absorbed NH₄OH in soils*

SOIL	MgO OR NaOH	0.1 N TITER OF 100 CC. DISTILLATES						
		1	2	3	4	5	6	SUM 1-2
Dewey silt loam	MgO*	10.1	.3					10.3
	NaOH†	10.1	.2					10.3
Decatur silt loam	MgO	13.4	.5	.1	.1			13.4
	NaOH	14.3	.2	.1	.1			14.5
Colbert silty clay loam	MgO	14.3	1.0	.5	.3			15.3
	NaOH	18.5	.3	.1	.1			18.8
Colbert silty clay loam subsoil	MgO	30.9	2.6	1.4	.8	.6	.4	33.5
	NaOH	38.2	.6	.1	.1			38.8
Colbert silty clay loam X-10	MgO	35.2	2.2	1.2	1.0	.6	.5	37.4
	NaOH	44.6	.7	.2	.1	.1		45.3
Becket sandy loam	MgO	24.7	.4	.15	.15			25.1
	NaOH	25.4	.4	.20	.15			25.8

* 0.5 gram of MgO for each determination.
† 12.5 cc. normal NaOH determination.

It was anticipated that objection would be raised against the use of sodium hydroxide on the grounds that it decomposes soil organic matter. Sodium hydroxide effects a dispersion of organic matter, but the results of Table 3 show no vitiative effect on the absorbed ammonium determination, since the higher values obtained by the use of sodium hydroxide are not coincident with the high organic matter content of the soils. On the contrary, the greatest differences occur with soils of low organic matter content and subsoils. The minimal difference between the ammonia releases by magnesium oxide and sodium hydroxide was shown by the Becket sandy loam, whose absorption is due almost entirely to the organic matter content. Moreover, on soils with only moderate absorption capacity, 10 m.e. or less, concordance in ammonia distillations is shown for the two hydroxides. With soils of high inorganic colloid content, however, complete recovery of the absorbed ammonia is not effected by distillation with magnesium oxide.

EFFECT OF AMMONIA ADDITIONS TO THE AMMONIUM CHLORIDE SOIL SUSPENSION AND TO ALCOHOL WASH ON EXCHANGE CAPACITY VALUES

Neutralization of the wash alcohol with ammonium hydroxide is generally followed by investigators in determining exchange capacity by ammonia absorption. It was found difficult to maintain the alcohol at the neutral point and the quantities of ammonia required for neutralization appeared to be excessive. Furthermore, the addition of the slightest amount of water to such neutralized alcohol results in a pH value of 8 or 9. Usually about 2.5 cc. of normal ammonium hydroxide per liter of alcohol is required to raise the pH to 7.0, but the addition of only 0.1 cc. of 0.1 N sodium hydroxide to 100 cc. of alcohol brought a pH of 9.0. This problem is also linked up with the state of ammonia saturation of the soil after digestion. Because of the hydrolysis of the ammonium chloride the soil suspension after digestion is distinctly acid, pH of about 4.0. This acid condition is neutralized by the addition of 15 cc. of 0.1 N ammonium hydroxide to the soil suspension before filtering. It is probable that this constant addition of ammonia will not effect the same degree of neutralization of the various soil-ammonium chloride systems. Within a wide range of soils it is possible that some may not be completely neutralized, whereas others may be by the 15 cc. addition of 0.1 N ammonium hydroxide. To evaluate the effect of varying degrees of neutralization of the soil suspension subsequent to the digestion with the ammonium chloride, absorbed ammonia was determined on three typical soils under different conditions as to neutralization of the soil suspension and also the alcohol wash. The experimental results are given in Table 4.

In considering these data the maximal ammonia absorption was taken as the basis of comparison for the various treatments. In treatment A, in which the soil suspension was filtered without neutralization and washed with untreated alcohol, the ammonia retentions were from 52 to 84 per cent of the respective maxima. The greatest variation from maximum, as a result of withholding of ammonia from both stages of the procedure, is shown for the soils of high content of organic matter. The absorptive capacity of the Becket soil is due almost entirely to its organic matter content, and the greatest deficiency occurred in this soil. Treatment B, in which the soil suspensions were not neutralized, but in which the alcohol wash contained the equivalence of 5 cc. of 0.1 N ammonium hydroxide, caused an ammonia absorption practically equal to the ammonia carried by the alcohol wash. This indicates that the alcohol readily yields its ammonia content to the unsaturated soils. Treatment C, in which the soil suspensions were neutralized, but which were washed with plain alcohol, was not quite so effective as treatment B in the build-up of ammonia absorptions. This means that the absorption from 5 cc. of 1 N ammonia in the alcohol wash was greater than absorptions

TABLE 4.—*Effect of NH₄OH additions to soil suspension and to alcohol wash, upon exchange capacity value by the NH₄Cl method*

SOIL	SERIES	0.1 N NH₄OH ADDED TO—		EXCHANGE CAPACITY
		SUSPENSION	ALCOHOL	
		cc.	*cc.*	*m.e.*
Colbert silty clay subsoil	A	0	0	31.6
	B	0	5	35.5
	C	15	0	34.5
	D	15	5	36.2
	E	15	12.5	37.8
	F	25	5	36.8
Colbert silty clay loam No. 6561	A	0	0	25.2
	B	0	5	30.3
	C	15	0	29.8
	D	15	5	32.8
	E	15	12.5	33.9
	F	25	5	32.4
Becket sandy loam (very high organic matter content)	A	0	0	25.0
	B	0	5	33.6
	C	20	0	32.0
	D	20	5	39.7
	E	20	12.5	48.0
	F	30	5	45.8

from additions of 10–20 cc. to the soil suspensions. Treatment D, whereby both the soil suspensions and the alcohol wash were neutralized, registered ammonia absorptions close to the maximum for the two minerals soils against a disparity of about 8 m.e. for the Becket sandy loam, which is essentially an organic complex. Treatment F, in which an extra 10 cc. of ammonia was added to the soil suspensions and which otherwise was the same as treatment D, gave results almost identical to those of the soils to which no excess of ammonia was added, except for the Becket soil, which showed an appreciable increase in absorbed ammonia.

The results of these experiments indicate that (a) neutralization of the soil suspension is essential only to an approximate degree, (b) ammoniation of the alcohol wash has a balancing effect in removing excess of ammonia as well as in neutralizing absorbed hydrogen in the soil system, (c) increases in the ammonia content of the alcohol wash exerts only a slight effect on ammonia absorbed by mineral soils, such increase causing marked enhancement in ammonia absorption by soils of high organic matter content.

It is recommended[1] that studies of the function of ammonia in the alcohol wash used in the determination of base exchange capacity be continued.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 53 (1939).

REPORT ON LESS COMMON ELEMENTS IN SOILS
FLUORINE IN SOIL AND OTHER MATERIALS RELATED TO AGRICULTURE

By J. S. McHARGUE, *Associate Referee,* and W. S. HODGKISS
(Department of Chemistry, Kentucky Agricultural Experiment Station, Lexington, Ky.)

During the past year the Department of Chemistry of the Kentucky Agricultural Experiment Station has been interested in ascertaining the fluorine content of soil and other materials related to agriculture. The analytical results reported here were obtained by W. S. Hodgkiss, and collaborative work was accomplished through the assistance of W. H. MacIntire and J. W. Hammond of the Tennessee Experiment Station, who sent to this laboratory three samples of soil and two samples of bones for analysis of their fluorine content, and also a copy of the methods they use for the determination of the fluorine content of organics, siliceous materials, and soils.

These methods were followed in the determinations of fluorine in the samples submitted. Some difficulty was experienced in checking some of the results on fluorine sent to us later by Hammond, but with further experience, significant differences in the results obtained by the two collaborators were largely eliminated. Table 1 shows the results obtained by Hammond and Hodgkiss.

TABLE 1.—*Results obtained by Hammond and Hodgkiss*

	FLUORINE CONTENT	
	HAMMOND	HODGKISS
Tennessee Samples		
	p.p.m.	
Lysimeter Soil No. 1	110	83
Lysimeter Soil No. 2	743	411
Red Clay Subsoil No. 3	9	45
Steamed Bone A	920	977
Normal Bone B	404	428
Kentucky Samples		
Sandy loam soil, Ky. C-4165	0.59	0.68
Wheat, plot No. 1, above soil, grain	1.80	1.85
Wheat, plot No. 1, above soil, straw	0.72	0.84
Wheat, plot No. 12 P.C., above soil, grain	1.39	1.24
Wheat, plot No. 12 P.C., above soil, straw	0.72	0.61
Wheat, plot No. 13 R.P., above soil, grain	1.40	1.76
Wheat, plot No. 13 R.P., above soil, straw	0.84	0.63
Phosphatic Sand (B.P.L. 60%)	3.26%	3.28%

During the year this Department was also called upon to make fluorine determinations of about 25 samples of bones collected from horses ranging in age from suckling colts to animals five or six years old. The

fluorine content of the samples ranged from about 100 p.p.m. in the fat-free bones of the younger animals to approximately 300 p.p.m. in similar fat-free bones of the mature horses.

In the course of the analyses for fluorine in the bones of the horses it was observed that when a sample of raw bone was burned in a silica dish with a Bunsen burner until all the volatile matter was consumed and the

TABLE 2.—*Fluorine content of ashed whole bone as compared to fat-free bone*
(1 gram sample used)

LAB. NO.	KIND OF BONE	F FAT-FREE BONE ASH BASIS	F WHOLE BONE ASH BASIS
		p.p.m.	*p.p.m.*
C-10053	Rib	246	254
C-10054	Vertebrae	313	310
C-10055	Femur	276	318
C-10060	Rib	228	263
C-10061	Vertebrae	235	233
C-10062	Femur	241	246

incineration was continued in a muffle furnace at dull red heat until free of carbon, the bone ash thus obtained contained as much fluorine as did the ash in other portions of the same sample which were extracted with ether to remove the fat.

This observation suggested the following experiments: Fat-free bone samples were ashed without the addition of magnesium peroxide, which is

TABLE 3.—*Fluorine content of fat-free bones from the same animal ignited at 600° C. without and with the addition of magnesium peroxide*
(1 gram sample used)

LAB. NO.	KIND OF BONE	MgO₂ ADDED	FLUORINE
		gram	*p.p.m.*
C-10053	Ribs	0.0	148.2
C-10053	Ribs	0.5	146.0
C-10053	Ribs	1.0	148.0
		Av.	147.4
C-10054	Vertebrae	0.0	174.0
C-10054	Vertebrae	0.5	173.4
C-10054	Vertebrae	1.0	175.0
		Av.	174.1
C-10055	Femur	0.0	164.0
C-10055	Femur	0.5	163.5
C-10055	Femur	1.0	165.0
		Av.	164.2

a slight modification of the procedure suggested by MacIntire and Hammond for the determination of fluorine in materials containing organic matter.

The results (Table 3) indicate that it is not necessary to add magnesium peroxide in the determination of fluorine in fat-free bone material when the samples are burned for about 3 hours in an electric furnace at approximately 600° C.

The slight modifications in the method for the determination of fluorine in bones suggested by the results of the foregoing experiments probably apply only to bones. No other experiments were made to ascertain whether other materials containing organic matter can be burned directly without the loss of fluorine.

Other products that have required the determination of the fluorine content during the past year are the commercial mineral mixtures that are used as supplements to the feed for live stock.

TABLE 4.—*Fluorine content of mineral mixtures used for live stock*

SAMPLE	per cent
1	0.0326
2	0.0386
3	0.0430
4	0.0578
5	0.1157
6	0.1748
7	0.3550
8	0.5220
9	0.7147

Due to the rather high percentages of fluorine contained in certain of these mineral mixtures and also to the fact that a considerable number of farmers in Kentucky have experienced the loss of valuable live stock from their use, the Departments of Chemistry and Feed Control of the Kentucky Agricultural Experiment Station regard mineral mixtures containing as much fluorine as the majority of the above samples show as a potential source of danger to live stock. The importance of a reliable method for the determination of fluorine in products related to agriculture is therefore apparent.

It is recommended[1] that further study be made of the calcium peroxide method for the determination of fluorine in soil. It is suggested that comparative data be obtained on the fluorine content of soil by its determination by fusion with sodium or potassium hydroxide in a nickel crucible and the results obtained by the calcium peroxide procedure.

No report on selenium in soils was given by the associate referee.

[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 53 (1939).

The two papers on the "Decomposition of Dolomite Limestone in Soils when Used as Neutralizing Agents in Complete Fertilizers," presented by Dawson, Snyder, Leighty, and Reid, and by Collins and Speer, respectively, were published in *This Journal*, 22, 137, 142 (1939).

REPORT ON FERTILIZERS

By G. S. FRAPS (Agricultural and Mechanical College of Texas, College Station, Texas), *Referee*

The recommendations of the various associate referees will be presented in their reports, and therefore the Referee will do no more here than to express the appreciation of this Association for their services. There are, however, two subjects that should be discussed.

A recent method by one of the members of this Association requires the use of wide-necked graduated flasks of suitable size to take a two-holed rubber stopper. In response to a letter protesting the use of such inaccurate measuring devices, he replied that flasks of this kind are listed by chemical supply houses as "fertilizer flasks." The diameters of the necks of such flasks are far beyond the sizes adopted by the Bureau of Standards. The use of such flasks brings up the question whether a certain degree of accuracy should not be required in the weights, volumetric flasks, pipets, and burets, used in connection with A.O.A.C. methods of analysis. The Bureau of Standards has set limits of precision for measuring instruments and for weights. These limits may be too exacting for control work but if so, limits of error should be adopted so that the use of inaccurate measuring instruments would not be permitted by this Association. It is not difficult to test the calibration of volumetric flasks by means of a Morse buret calibrated by the Bureau of Standards, and pipets can be tested by weighing the water which they deliver. The testing of burets is somewhat more difficult. At this Station the pipets and measuring flasks have been tested for a number of years. Recently 21 of 24 graduated flasks manufactured by a national manufacturer, did not meet the requirements and were returned. Two or three years earlier another lot of 20 out of 24 was returned as not sufficiently accurate. It is not unusual to receive a few flasks and pipets that are not sufficiently accurate. Weights, though accurate when purchased, may deteriorate under the severe service of a control laboratory, and may need testing from time to time.

The Referee believes that this Association should consider this matter and specify the use of instruments of the proper degree of exactness for control work, which would at the same time outlaw the wide-necked flasks now known as fertilizer flasks and other inaccurate instruments. Therefore it is recommended that an associate referee be appointed to

test volumetric instruments and weights and to work in cooperation with the Bureau of Standards.

At the last meeting of the Association a method for estimating the available phosphoric acid in fertilizers was presented, and it has since been published in *The Journal* of this Association and in *Industrial and Engineering Chemistry*. The official ammonium citrate method for determining available phosphoric acid has been in use for over 50 years and is the basis for large transactions in phosphates. It is therefore necessary for this Association to examine very carefully and thoroughly any new method in order to see that it meets the requirements of the trade and does not bring about any unforeseen and undesirable consequences. The proposed new method is probably shorter than the one now in use, provided the determination of total phosphoric acid is not needed, since the new method requires only one determination of phosphoric acid instead of two. The new method is claimed to give the same results with superphosphate as does the present method, but the analyses given in the paper referred to are not altogether in accord with this claim, since one of them is 0.8 per cent lower than the official method, and other differences are shown. Analyses of 18 samples were made by the proposed method in the Texas Laboratory by S. E. Asbury. Of 49 determinations 12 (nearly 25 per cent) were discarded as too wide. The new method did not give entirely concordant results on the same samples. When the average results are compared, 7 come within 0.25 per cent of the official method, one is 0.25–.40 per cent too low, 4 are over 0.4 per cent too low, 2 are 0.25–0.4 too high, and 4 are over 0.4 per cent too high. The new proposed method thus does not give exactly the same results on superphosphate as the official method. It is necessary to consider seriously the question whether the different results secured by the new method with some superphosphates will offset the possible advantages of the method. There should also be some reason given for the discrepancies between the results of the two methods. The proposed method also needs modification so as to secure concordant results on the same samples. The pH of the solvent prepared by the method described is 4.4 and not 4.2, as stated in the description. This difference requires explanation.

The proposed new method also gives different results from the official method with other materials than superphosphate, and in some cases the differences are quite large. Much more of the phosphoric acid of tricalcium phosphate is found to be available by the new method than by the official method. What effect these differences would have on the fertilizer industry is a matter for consideration. The new method is worthy of study, but many comparisons with the official method among fertilizers are needed, together with much information regarding the reasons for the differences which will be found, before the Association will be in position

to consider seriously the question of replacing the official method by the new method.

RECOMMENDATIONS[1]

The Referee on Fertilizers makes only one recommendation in addition to those made by the associate referees, namely, that an Associate Referee on Testing Volumetric Apparatus and Weights be appointed, to work in cooperation with the Bureau of Standards and to recommend methods for discouraging the use of apparatus and weights that are too inaccurate.

REPORT ON PHOSPHORIC ACID

A. EFFECT OF METHOD OF FILTERING ON DETERMINATION OF WATER-SOLUBLE P_2O_5. II.
B. VARIATION IN CITRATE-INSOLUBLE P_2O_5 WITH TIME INTERVAL BETWEEN WATER EXTRACTION AND CITRATE DIGESTION, II.

By William H. Ross, *Associate Referee*, and J. Richard Adams
(Bureau of Chemistry and Soils, Washington, D. C.)

In a laboratory study that was made last year by Adams and Ross[2] at the suggestion of the Referee on Fertilizers,[3] it was found that:—

(A) The kind of filter paper on which the sample is washed in the determination of water-soluble P_2O_5 has little, if any, effect on the results, and that washing with suction gives lower results than washing under gravity.

(B) The citrate-insoluble P_2O_5 in materials such as ammoniated superphosphate, which contain di- or tricalcium phosphate, increases with prolonged standing of the washed residue before digestion in citrate solution, but not in materials, such as ordinary superphosphate, which do not contain appreciable quantities of either di- or tricalcium phosphate.

A recommendation was adopted at the last meeting of this Association that the work be continued by collaborative study. The standard samples submitted to the collaborators in compliance with this recommendation were as follows:

STANDARD SAMPLES

1. Mixed fertilizer (6-12-6).
2. Mixed fertilizer (5-10-5).
3. A mixture of the principal components of an ammoniated superphosphate, fluorine free.
4. A mixture of the principal components of an ammoniated superphosphate, containing 2.4 per cent of fluorine.
The formulas of the mixed fertilizer samples were as follows:

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 51 (1939).
[2] *This Journal*, 21, 268 (1938).
[3] *Ibid.*, 20, 228 (1937).

MATERIAL	MIXED FERTILIZER	
	(6-12-6) SAMPLE NO. 1	(5-10-5) SAMPLE NO. 2
Ammoniated superphosphate (5.65% N; 11.33% avail. P_2O_5)	1420	—
Ordinary superphosphate (20.25% avail. P_2O_5)	—	989
Dicalcium phosphate (51.37% avail. P_2O_5)	154	—
Ammonium sulfate (20.9% N)	97	383
Sodium nitrate (16.5% N)	121	121
Potassium chloride (57.8% K_2O)	208	173
Tennessee rock phosphate	—	334
	2000	2000

The compositions of the assimilated ammoniated superphosphate samples were as follows:

MATERIAL	ASSIMILATED AMMONIATED SUPERPHOSPHATE	
	SAMPLE NO 3	SAMPLE NO. 4
Monoammonium phosphate	100	100
Dicalcium phosphate	1100	1100
Tricalcium phosphate	500	500
Ammonium sulfate	200	200
Calcium sulfate dihydrate	100	—
Calcium fluoride	—	100
	2000	2000

Samples 1 and 4 contained fluorine and di- and tricalcium phosphates; Sample 2 contained fluorine but no di- or tricalcium phosphate; while Sample 3 contained di- and tricalcium phosphate but no fluorine.

The directions sent to the collaborators were as follows.

DIRECTIONS FOR ANALYSIS

A-1. Determine water-soluble P_2O_5 in each of the standard samples as directed in *Methods of Analysis*, A.O.A.C., 1935, p. 21, **13**, using a 9 cm. Whatman filter No. 2 for the water extraction, and wash under gravity.

A-2. Repeat the determinations, using a 9 cm. Whatman filter No. 5, and wash under gravity.

A-3. Repeat the determinations, using a 9 cm. Whatman filter No. 5, and wash under suction.

A-4. Repeat the determinations, using a Shimer filter, and wash under suction.

B-1. Determine citrate-insoluble P_2O_5 in each of the standard samples as directed in *Methods of Analysis*, A.O.A.C., 1935, for acidulated samples, p. 22, **16(a)**. In making these determinations, wash the sample by one of the procedures outlined above, and when the washing has been completed, immediately transfer the filter containing the residue to the citrate solution at 65° C.

B-2. Repeat the determinations, allowing the washed residue to stand on the filter paper for 2 hours before transferring it to the citrate solution.

B-3. Repeat the determinations, allowing the washed residue to stand on the filter paper for 4 hours before transferring it to the citrate solution.

B-4. Repeat the determinations, allowing the washed residue to stand on the filter paper for 18 hours before transferring it to the citrate solution.

NOTES

a. The filter pulp for the Shimer filter is most conveniently prepared from Schleicher and Schüll's No. 292 filter pulp disks. When these are not available, prepare the filter pulp by tearing from five to eight 9 cm. filters or their equivalent of sheet filters into shreds, place the pieces in a 500 cc. Erlenmeyer flask, add 250 cc. of hot water, close the flask with a rubber stopper, and, under the protection of a towel, shake vigorously until the paper is reduced to a pulp. The mat in the Shimer filter should be $\frac{1}{4}''-\frac{3}{8}''$ thick when compacted by suction. It is necessary to compact the filter mat by suction in order to make it sufficiently retentive to hold the residue, and to prevent disruption of its upper surface when the wash water or solution to be filtered is poured directly on the filter mat. A rubber stopper fastened to a glass rod may be used to compact the filter.[4]

b. The funnels in which the filters containing the washed residues are allowed to stand for a time before digestion in the citrate solution should be covered with a watch-glass and the tips of the funnels kept under water to prevent drying of the residues.

c. The results obtained for each sample by the different procedures outlined should not vary greatly, and it is therefore very important, if the true effect of each of the factors under investigation is to be determined, that no variation be made in any of the procedures other than the one specified.

METHODS OF FILTERING

The directions submitted to each collaborator for the analysis of the standard samples were accompanied by a questionnaire requesting information on the method of filtering and the kind of filter used (a) in the determination of water-soluble P_2O_5; (b) in filtering off the yellow ammonium phosphomolybdate precipitate obtained in the volumetric method of determining P_2O_5; and (c) in filtering off the residue from the citrate digestion.

The replies received indicate that water-soluble P_2O_5 is commonly determined by washing on filter paper under gravity. The method of filtering the phosphomolybdate precipitate, however, varies greatly in different laboratories. The preferred procedure seems to consist in filtering on a mat of asbestos or filter paper pulp in a Shimer or similar type filter. Filtering with suction on a filter mat was reported by C. A. Butt to have advantages over filter paper in the ordinary funnel in that it requires a smaller number of washings for complete removal of acidity, decreases danger of loss of precipitate, and avoids the necessity of pulping the filter paper before titration.

The relative efficiency of filter paper and of asbestos mats in filtering

⁴ MacIntire, Jones, and Hardin, *This Journal*, 18, 301 (1935).

ammonium phosphomolybdate precipitates was compared by one of the writers in the analysis of a standard solution of monopotassium phosphate by the volumetric method. The range of the results obtained with filter paper, filter pulp mats, and mats made from seven grades of asbestos, including recovered asbestos, did not exceed the limits of experimental error. This study also supported the claims of Butt as to the advantages of filter mats over filter paper in filtering the phosphomolybdate precipitates in routine work.

The method most commonly used by the collaborators for the recovery of the citrate-insoluble residues consists in filtering with suction on one or more layers of filter paper in a Büchner or glass funnel.

COLLABORATORS

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RESULTS OF ANALYSIS

Table 1 summarizes the results reported by the collaborators for water-soluble P_2O_5 in the standard samples by different methods of filtering. The results obtained for citrate-insoluble P_2O_5 in the samples when the time interval between washing and the citrate digestion was varied are given in Table 2.

INTERPRETATION OF RESULTS

The values given in Table 1 agree with the conclusions of last year's report[2] in showing (a) that the two types of filter paper used in determining water-soluble P_2O_5 have little or no effect on the results; and (b) that washing with suction gives lower results for water-soluble P_2O_5 than washing under gravity.

It is well known that the calcium phosphates, with the exception of the last in the series, hydrolyze in contact with water to form a less soluble phosphate and free phosphoric acid as illustrated in the following equation:

$$10CaHPO_4 + 2H_2O = [3Ca_3(PO_4)_2] \cdot Ca(OH)_2 + 4H_3PO_4. \qquad (1)$$

Slow washing promotes hydrolysis, and the higher results obtained for water-soluble P_2O_5 by gravity washing as compared with washing by suction are no doubt due to the greater proportion of free phosphoric acid formed during the slower washing procedure. Hydrolysis as a result of slow washing may also give rise, in the presence of fluorine, to an increase

TABLE 1.—*Effect of method of filtering on water-soluble P_2O_5 in standard phosphate samples*

	PER CENT WATER-SOLUBLE P_2O_5 BY FILTRATION UNDER—			
	GRAVITY		SUCTION	
COLLABORATOR	WHATMAN FILTER		WHATMAN FILTER	SHIMER FILTER
	NO. 2	NO. 5	NO. 5	
	Sample No. 1			
1	2.96	3.19	2.68	2.34
2	3.00	3.08	2.66	2.83
3	3.29	3.21	2.58	2.36
4	3.03	3.13	2.75	2.64
5	3.13	3.16	3.19	3.07
6	3.07	3.17	2.45	2.53
7	4.00	3.13	2.92	2.83
8	3.22	3.18	3.00	2.80
9	3.36	3.32	3.16	3.00
10	3.42	3.45	3.32	2.97
11	3.15	3.30	2.90	2.90
12	2.98	2.98	2.90	2.80
13	3.05	3.00	2.58	2.60
14	3.15	3.20	2.90	2.90
15	3.18	3.01	2.90	2.73
Mean	3.20	3.17	2.85	2.75
	Sample No. 2			
1	8.95	9.26	8.93	8.90
2	8.68	8.77	8.53	8.73
3	9.00	9.09	8.55	8.60
4	8.77	8.87	8.48	8.58
5	9.08	9.06	9.09	9.02
6	9.05	9.04	8.38	8.48
7	9.18	9.12	9.01	8.98
8	9.02	8.89	8.68	8.57
9	8.86	8.92	8.90	8.95
10	9.27	8.95	9.22	8.98
11	9.20	9.30	8.85	9.00
12	8.85	8.88	8.80	8.83
13	8.70	8.70	8.50	8.43
14	9.15	9.25	8.85	8.85
15	9.19	9.09	8.89	8.64
Mean	8.99	9.01	8.78	8.77

TABLE 1.—*Effect of method of filtering on water-soluble P_2O_5 in standard phosphate samples—Continued*

	PER CENT WATER-SOLUBLE P_2O_5 BY FILTRATION UNDER—			
COLLABORATOR	GRAVITY		SUCTION	
	WHATMAN FILTER		WHATMAN FILTER	SHIMER FILTER
	NO. 2	NO. 5	NO. 5	
		Sample No. 3		
1	3.51	3.70	3.38	3.28
2	3.30	3.36	3.09	3.19
3	3.12	3.15	2.80	2.93
4	3.57	3.62	3.49	3.34
5	3.70	3.75	3.67	3.70
6	3.65	3.65	2.78	2.78
7	3.81	3.72	3.47	3 50
8	3.65	3.58	3.45	3.30
9	3.48	3.60	3.54	3.30
10	3.50	3.45	3.77	3.52
11	3.80	3.80	3.50	3.55
12	3.53	3.45	3.48	3.38
13	3.20	3 85	3.08	3.05
14	3.80	3.80	3 40	3.45
15	3.29	3.03	3.31	3.21
Mean	3.52	3.57	3.35	3.30
		Sample No. 4		
1	4.07	4.24	3.78	3.60
2	4.11	4.22	3.70	3.82
3	4.06	4.13	3.50	3.60
4	4.13	4.30	3.79	3.85
5	4.20	4.24	4.23	4.17
6	4.40	4.42	3.42	3.38
7	4.40	4.00	3.90	4.06
8	4.23	4.19	3.97	3.90
9	4.40	4.56	4 08	4.12
10	4.15	4.60	4.17	3.92
11	4.40	4.50	3.90	3.90
12	4.20	4.28	3.95	3.98
13	3.95	3.95	3.73	3.68
14	4.25	4.25	3.85	3.90
15	4.20	3.98	4.06	3.84
Mean	4.21	4.26	3.87	3.85

in citrate-insoluble P_2O_5, as shown in the paper by Rader and Ross.[5] It would seem, therefore, that the determination of water-soluble P_2O_5 should be made as quickly as possible and that washing with suction should be recommended for samples that can not be rapidly washed under gravity.

[5] *This Journal*, 22, 400 (1939).

The results given in Table 2 are also in agreement with the conclusion reached last year, that prolonged standing of the washed residue before digestion in citrate solution causes an increase in citrate-insoluble P_2O_5 in the analysis of ammoniated mixtures of the ordinary type (Sample 1) but not in non-ammoniated mixtures (Sample 2).

In the ammoniation of fertilizer mixtures the monocalcium phosphate of the superphosphate in the mixture is changed into monoammonium

TABLE 2.—*Effect of varying the time interval between washing and citrate digestion on the citrate-insoluble P_2O_5 in the standard phosphate samples*

COLLABORATOR	PER CENT CITRATE-INSOLUBLE P_2O_5 WITH TIME INTERVALS BETWEEN WASHING AND CITRATE DIGESTION OF—			
	0 hours	2 hours	4 hours	18 hours
	Sample No. 1			
1	3.31	3.43	3.45	3.79
2	3.38	3.42	3.54	3.88
3	2.98	3.00	3.14	3.20
4	3.01	3.32	3.10	3.76
5	3.65	3.64	3.66	3.80
6	2.70	3.15	3.40	3.65
7	4.00	4.50	4.30	4.25
8	3.61	3.70	3.74	3.90
9	2.21	2.10	2.40	2.57
10	3.38	3.39	3.43	3.88
11	3.90	4.70	4.75	4.70
12	3.10	3.26	3.17	3.51
13	2.60	2.74	2.90	2.94
14	3.80	3.95	4.00	4.00
15	2.43	2.55	2.61	2.83
Mean	3.19	3.39	3.44	3.64
	Sample No. 2			
1	4.66	4.73	4.66	4.70
2	4.67	4.62	4.70	4.69
3	4.50	4.50	4.36	4.53
4	4.67	4.56	4.51	4.55
5	4.92	4.90	4.88	4.92
6	4.68	4.53	4.55	4.58
7	4.60	4.52	4.60	4.65
8	4.79	4.79	4.81	4.70
9	4.47	4.41	4.42	4.53
10	4.77	4.77	4.91	4.97
11	4.75	4.75	4.75	4.90
12	4.75	4.76	4.77	4.75
13	4.46	4.44	4.38	4.40
14	4.70	4.75	4.80	4.85
15	4.51	4.61	4.49	4.49
Mean	4.66	4.64	4.64	4.68

TABLE 2.—*Effect of varying the time interval between washing and citrate digestion on the citrate-insoluble P_2O_5 in the standard phosphate samples—Continued*

COLLABORATOR	PER CENT CITRATE-INSOLUBLE P_2O_5 WITH TIME INTERVALS BETWEEN WASHING AND CITRATE DIGESTION OF—			
	0 hours	*2 hours*	*4 hours*	*18 hours*
		Sample No. 3		
1	2.31	2.19	2.28	2.61
2	2.38	2.39	2.42	2.39
3	1.91	1.86	2.00	2.10
4	2.50	2.59	2.60	2.64
5	2.78	2.74	2.73	2.85
6	2.40	2.15	2.20	2.45
7	3.15	3.16	3.37	3.00
8	2.76	2.74	2.74	2.77
9	1.49	1.68	1.61	2.03
10	2.41	2.26	2.36	2.43
11	3.15	3.25	3.25	3.45
12	2.39	2.37	2.34	2.52
13	1.86	1.98	2.00	1.82
14	2.45	2.65	2.70	2.75
15	1.72	1.66	1.84	1.88
Mean	2.38	2.38	2.43	2.51
		Sample No. 4		
1	2.67	2.94	2.96	3.30
2	3.62	3.72	3.79	4.37
3	2.85	2.90	3.08	3.19
4	3.18	3.30	3.31	3.40
5	3.63	3.67	3.68	3.74
6	2.23	2.65	2.70	3.30
7	3.40	3.54	4.25	3.95
8	3.68	3.70	3.69	4.07
9	2.27	2.59	2.33	3.13
10	3.10	3.08	3.31	4.02
11	3.80	4.00	4.15	4.40
12	2.82	2.97	2.95	3.10
13	2.82	2.60	3.00	2.96
14	3.20	3.55	3.70	4.00
15	2.22	2.47	2.79	2.68
Mean	3.03	3.18	3.31	3.57

phosphate, and di- or di- and tricalcium phosphates.[6,7] Sample 3 contained these three compounds and calcium sulfate. Sample 4 differed from Sample 3 only in that the calcium sulfate was replaced with calcium fluoride. The results in Table 2 show that little, if any, increase in citrate-insoluble P_2O_5 took place in Sample 3 when its washed residue was allowed

[6] Keenen, F. G., *Ind. Eng. Chem.*, **22**, 1378 (1930).
[7] White, Hardesty, and Ross, *Ibid.*, **27**, 562 (1935).

to stand for 18 hours before digestion, whereas the increase in citrate-insoluble P_2O_5 that occurred under the same conditions in the analysis of Sample 4 was about the same as in the case of Sample 1. It may, therefore, be concluded that the formation of citrate-insoluble P_2O_5 on prolonged standing of the washed residue before digestion in citrate solution is limited to samples that contain both fluorine and di- or tricalcium phosphate and that the decrease in available P_2O_5 in such samples is due to the formation of a relatively insoluble fluorine-containing phosphate,[8,9] as explained in the report of last year.[2]

Samples 3 and 4 contained the same phosphatic materials in exactly the same proportions. The total P_2O_5 in each sample amounted to 40.18 per cent. The initial water-soluble and citrate-insoluble P_2O_5 in the samples must also have been the same if the influence of the calcium fluoride on the solubility of the phosphates in Sample 4 is assumed to be the same as that of the calcium sulfate in Sample 3. The results reported by the collaborators, however, show a higher value for both water-soluble and citrate-insoluble P_2O_5 in Sample 4 than in Sample 3. These results would be expected if the reactions that may have taken place during the storage and analysis of Sample 3 were accompanied in Sample 4 by the additional reaction represented in Equation 2.

$$9CaHPO_4 + CaF_2 = [3Ca_3(PO_4)_2] \cdot CaF_2 + 3H_3PO_4. \qquad (2)$$
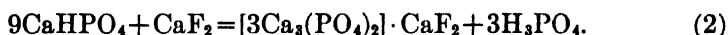
Results obtained by Rader and Ross[5] in a study of the reversion of P_2O_5 during analysis not only conform with the reports of the collaborators, but they also show that an increase in citrate-insoluble P_2O_5 usually accompanies a prolonged digestion of the washed residue in citrate solution. They show, moreover, that little or no reversion occurs during analysis of cured mixtures when each of the different steps in the process is completed within one hour, and that the increased citrate-insoluble P_2O_5 found in Sample 4 as compared with Sample 3 occurred during storage of the sample before analysis rather than during the process of analysis.

CONCLUSIONS

Slow washing of fertilizer mixtures of the ordinary type gives higher results for water-soluble P_2O_5 than does rapid washing due to the formation of free phosphoric acid by hydrolysis of the citrate-soluble phosphates present. If fluorine is present, the increase in water-soluble P_2O_5 may be accompanied by an increase in citrate-insoluble P_2O_5.

Prolonged standing of the washed residue before digestion in citrate solution causes an increase in citrate-insoluble P_2O_5 in ammoniated mixtures of the ordinary type, and in mixtures containing fluorine and di- or tricalcium phosphates. Prolonged standing of the washed residue has little

[8] MacIntire, Hardin, Oldham, and Hammond, *Ibid.*, 29, 758 (1937).
[9] Ross, Rader, and Beesom, *This Journal*, 21, 258 (1938).

if any effect on the citrate solubility of the P₂O₅ in fluorine-free mixtures or in non-ammoniated mixtures of the ordinary type. Little or no reversion occurs in the analysis of cured phosphatic materials or mixtures when each of the different steps in the process is completed within a period of one hour.

<div align="center">RECOMMENDATIONS[10]</div>

It is recommended—

(1) That the words, "Place 1 g of the sample on a 9 cm filter and wash with successive small portions of H₂O, allowing each portion to pass thru before adding more, until the filtrate measures about 250 cc," *Methods of Analysis, A.O.A.C.*, 1935, sec. **13**, p. 21, lines 1–3, be changed to read, "Place 1 g of the sample on a 9 cm filter and wash with successive small portions of H₂O until the filtrate measures about 250 cc. Allow each portion of the wash water to pass thru the filter before adding more, and wash with suction if the washing would not otherwise be complete within 1 hour."

(2) That the words, "Heat 100 cc of the NH₄ citrate soln to 65° in a 250 cc flask . . . Shake the flask every 5 min.," *Methods of Analysis, A.O.A.C.*, 1935, sec. **16(a)**, p. 22, lines 1–8, be changed to read, "After washing out the water-soluble P₂O₅, **13**, transfer the filter and residue, within a period not to exceed an hour, to a 250 cc flask containing 100 cc of the NH₄ citrate soln previously heated to 65° in a water bath. Close the flask tightly with a smooth rubber stopper and shake vigorously until the filter paper is reduced to a pulp, relieving the pressure by momentarily removing the stopper. Loosely stopper the flask to prevent evaporation and return it to the bath. Maintain the contents of the flask at exactly 65°, keeping the level of the H₂O in the bath above that of the citrate soln in the flask. Shake the flask every 5 min."

(3) That the Associate Referee give further consideration to the method proposed by MacIntire, Shaw, and Hardin[11] for the determination of available phosphoric acid.

<div align="center">

REPORT ON NITROGEN*

</div>

<div align="center">By A. L. PRINCE (Agricultural Experiment Station, New Brunswick, N. J.) *Associate Referee*</div>

The official method for determining water-insoluble nitrogen in organic materials is still open to a number of criticisms, despite the fact that certain minor changes have been made in an attempt to obtain more consistent results on all products. The recent changes, *This Journal*, **20**, 252 (1937), consisted in specifying the grade and size of filter paper to be

used on all materials. On the basis of the data obtained, these points were favorably passed upon as editorial changes at the 1936 meeting of the A.O.A.C.

Differences in results with various products still persist chiefly because of minor variations in the manner in which the method is run. Renewed interest in this subject has arisen from two sources: (1) certain States have passed laws which require a guarantee of the water-insoluble nitrogen; (2) a demand has been created for material showing a high percentage of water-insoluble nitrogen, and consequently the producers desire a method which will give the highest results. Of course, the A.O.A.C. can not and should not devise methods to favor or give advantage to any particular product. Suggestions by those in the fertilizer industry or others as to changes in the methods are always acceptable and should receive careful consideration provided they make for the improvement of the methods in general.

The inherent differences occurring in various products often make it difficult to prescribe a definite procedure for a particular determination that will work exactly alike for all. One is faced with the problem of devising a special method for every product, or by being satisfied with the slight differences that occur when a universal method is adopted. The present policy of the A.O.A.C. tends toward the elimination of many methods that are essentially the same. Occasionally, when a widely used product is so radically different from the general run of material that the prescribed official method gives widely divergent results, exception to the above policy should be allowed and a special method prescribed. This was the situation in the case of the determination of water-insoluble nitrogen in cyanamid. A special method for determining water-insoluble nitrogen in cyanamid was devised and finally adopted as an official method last year.

However, the majority of organic nitrogen materials are similar enough in character to warrant the use of a general method for determining water-insoluble nitrogen, provided the directions are made specific. Other factors beside the type of material which may cause discrepancies in the results by different analysts are the quality and size of filter paper, the manner of manipulation, and the temperature of the wash water. The first of these factors has been taken care of, namely, the quality and size of the filter paper. The second factor, manner of manipulation, refers to the actual method of washing and is probably the largest contributing factor toward disagreement in results among analysts. Unless the conditions of washing are definitely specified, analysts will vary their manipulation in respect to washing and obtain disagreeing results.

A preliminary study of this phase of the problem has been made during the past year. In washing various materials for water-insoluble nitrogen, it should be borne in mind that no substance is entirely insoluble, and that

prolonged washing would continually bring small amounts of the material into solution. It is therefore necessary to arbitrarily select a point where washing may be considered complete for all practical purposes.

Five different methods of washing were tried out on three materials. ground fish, process tankage, and peanut meal. The total nitrogen content of these materials was 8.11, 6.73, and 7.39 per cent, respectively. Both one and two gram samples were used, and each size sample was leached to two different volumes, namely, 200 cc. and 250 cc.

The different methods of washing are described below:

1. *Regular Official Method.*—Place the material on an 11 cm. Whatman No. 2 filter paper, wet with alcohol, and wash with water at room temperature to the desired volume. In this case the washing was rapid, new additions of water being added as soon as each portion went dry.

2. *Regular Method (modified).*—Same as No. 1, with the exception that the washing was carried on slowly, by allowing the funnel to drain 5 minutes between washings.

3. *Beaker Method.*—Place the material in a 50 cc. beaker, wet with alcohol, add 20 cc. of water, and allow the mixture to stand 15 minutes, with occasional stirring. Transfer the supernatant liquid to an 11 cm. Whatman No. 2 filter paper and wash 4 or 5 times by decantation with water at room temperature. Finally transfer all the residue to the filter paper and complete the washing rapidly to the desired volume.

4. *Shaking Method.*—Place the material in a 200–250 cc. Erlenmeyer flask and wet with alcohol; add 50 cc. of water, and place in a shaking apparatus. Shake for 15 minutes, then proceed as directed in the regular method.

5. *Automatic Method.*—

Apparatus: A 200 cc. volumetric flask containing a 2-holed rubber stopper. In each hole of the rubber stopper place two glass tubes, one a little shorter than the other (roughly 1½″, and 1¼″, respectively).

Method: Place the material on an 11 cm. Whatman No. 2 filter paper, wet with alcohol, and add 25 cc. of water. Immediately invert the volumetric flask containing water (25 cc. less than the required volume to which material is to be leached) over the paper with the tip of the longer tube just below the surface of the water in the paper. When the flask is empty, wash the paper with several small portions of water until the desired volume is reached.

The data obtained by these different methods for water-insoluble nitrogen in ground fish is reported in Table 1, and for process tankage and peanut meal, in Table 2. It will be noted that the automatic method gives relatively higher results than the other methods on all three materials, and also that there is a tendency for a greater divergence between individual determinations. Although this method is very rapid and is a great convenience as far as requiring attention, the results are too high and irregular.

The regular official method, specifying successive washing as soon as each portion goes dry, also runs higher in most cases than the slower filtration, beaker, or shaking methods. By this method, as well as the automatic method, excess water always remains on the material. The

TABLE 1.—*Per cent water-insoluble nitrogen in ground fish determined by different procedures*

METHOD	1 GRAM LEACHED TO 200 CC.	2 GRAMS LEACHED TO 200 CC.	1 GRAM LEACHED TO 250 CC.	2 GRAMS LEACHED TO 250 CC.
	per cent	*per cent*	*per cent*	*per cent*
Regular	6.02	5.94	5.73	5.81
	5.94	6.07	5.85	5.82
	6.06	5.98	5.72	5.89
Average	6.01	6.00	5.77	5.84
Regular (Modified)	5.85	5.88	5.77	5.73
	5.86	5.85	5.78	5.85
	5.86	5.89	5.80	5.78
Average	5.86	5.87	5.78	5.79
Beaker	5.94	5.90	5.80	5.81
	6.01	5.94	5.73	5.85
	5.99	6.00	5.80	
Average	5.98	5.95	5.78	5.83
Shaking	5.72	6.02	5.60	5.90
	5.77	6.02	5.81	5.83
	5.83	6.03	5.75	5.85
Average	5.77	6.03	5.73	5.86
Automatic	6.14	6.10	6.09	6.01
	6.23	5.64	6.02	5.90
	5.98	6.05	5.93	5.97
Average	6.12	5.93	6.02	5.96

data indicate that rapid washing on the funnel does not remove all the water-soluble nitrogen.

The regular method, modified to allow for slower filtration, checks quite closely with the beaker and shaking methods. The results by these three methods are quite comparable and the individual results are fairly consistent. However, the beaker method saves time in the filtration process, and allows at the start for thorough wetting and solubility of the material. B. F. Carpenter of the Virginia-Carolina Chemical Corporation also did considerable work on several types of material, using various methods of washing, and came to the conclusion that the beaker procedure was the best. It is a method that can be easily handled for rapid routine work.

The shaking method is also equally as effective with some materials such as with process tankage, but with certain materials, especially peanut meal, a colloidal suspension is obtained which prevents filtration. Consequently this method was eliminated in the peanut meal determinations.

Table 2.—*Water-insoluble nitrogen in process tankage and peanut meal by different procedures*

METHOD	PROCESS TANKAGE			PEANUT MEAL	
	1 GRAM LEACHED TO 200 CC.	2 GRAMS LEACHED TO 200 CC.	1 GRAM LEACHED TO 250 CC.	1 GRAM LEACHED TO 200 CC.	2 GRAMS LEACHED TO 200 CC.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Regular Official	5.98	5.86	6.04	2.65	2.43
Rapid Filtra-	6.09	5.99		2.71	2.43
tion	6.10				
Average	6.02	5.93	6.04	2.68	2.43
Regular Official	5.86	5.57	5.72	2.55	2.73
(Modified) Slow	5.80	5.60		2.52	2.75
Filtration	5.80				
Average	5.82	5.59	5.72	2.54	2.74
Beaker	5.77	5.80	5.62	3.02	2.62
	5.80	5.52		2.91	2.76
	5.81				
Average	5.79	5.66	5.62	2.97	2.69
Shaking	5.80	5.63	5.81		
	5.77	5.70			
	5.80				
Average	5.79	5.67	5.81		
Automatic	6.82	6.30	6.28	3.10	2.60
	6.85	6.36		3.23	2.70
	6.80				
Average	6.82	6.33	6.28	3.17	2.65

The problem of the formation of channels in washing the material by the regular, modified, or automatic methods, has a significant bearing on the results, but is of less importance with the beaker or shaking methods. The size and angle of the glass funnel is also an important factor in the filtration of the material. Long-stemmed funnels of 60° angle and having a diameter of 2½ inches in size allow for a more uniform rate of filtration.

The differences in results obtained when a one or two gram sample is used are not significant. Either weight when leached to the same volume appears to give comparable results except by the automatic method, where the results average lower when a 2 gram sample is used.

When the washing was carried on to a volume of 250 cc. rather than 200 cc., the results in general on ground fish and process tankage are slightly lower for water-insoluble nitrogen. In most cases the results are lower by between 0.1 and 0.2 of 1 per cent.

In the present official method for determining water-insoluble nitrogen,

Methods of Analysis, A.O.A.C., 1935, 28, 37, the material is washed to 250 cc. From the above data it would seem that this stipulation should continue, and all raw materials, such as fish tankage, cottonseed meal, etc., should be leached to this volume. The question is often raised as to why the weight of the sample and volume leached to should be different in the official method described under par. 34(b), page 27. Here, however, a preliminary determination is being made for the separation of different forms of nitrogen in a mixed fertilizer. The 2 gram charge taken and the subsequent 200 cc. volume obtained are very convenient quantities to work with for the remaining determinations. From the above data, and those obtained in 1937, *This Journal,* 20, 250–252, the differences brought about by the extra 50 cc. of wash water are not appreciable. Furthermore, the amount of organic material in mixed fertilizers is relatively small, and consequently less washing would be required.

Another point that has been raised concerning the manipulative features of this determination is the temperature of the wash water. The present directions specify room temperature, but this terminology covers quite a range, especially during winter and summer months, and in different parts of the country. Some analysts have already found differences as great as 0.3 of 1 per cent on samples washed with water at 31° C. in comparison with water used at 20° C. Hence it would seem wise to limit the term "room temperature" to a definite range, such as between 20°–25° C.

Although the beaker method seemed to yield the best results in this preliminary study, collaborative work should be done to compare the method with other procedures. The exact directions for this method are as follows: Place 1 or 1.4 grams of the material in a 50 cc. beaker, wet with alcohol, add 20 cc. of water, and allow to stand 15 minutes, with occasional stirring. Transfer the supernatant liquid to an 11 cm. Whatman No. 2 filter paper, and wash 4 or 5 times by decantation with water at room temperature (20°–25° C.). Long-stemmed funnels 2½ inches in diameter and having an angle of 60° should be used. Finally transfer all the residue to the filter paper and complete the washing until the filtrate measures 250 cc. Dry, and determine nitrogen in the residue as directed under 21 or 23.

During the past year attention was called to the fact that considerable difficulty was encountered in securing concordant results in the determination of total nitrogen in certain fish products, especially Canadian dogfish meal, by the regular A.O.A.C. methods. Consistent results were obtained, however, when a small percentage of potassium persulfate was incorporated in the digestion materials of the regular Kjeldahl-Gunning method. Ten grams of the following mixture was used for each determination: K_2SO_4, 83.7%; $K_2S_2O_8$, 9.3%; HgO, 7.0%. This modified procedure was tried out on a number of organic materials for comparison with the

regular method. The following substances were analyzed by both procedures: Crab meal, cottonseed meal, castor pomace, process tankage, garbage tankage, chicken manure, sewage sludge, and ground fish. Unfortunately, it was impossible to obtain any Canadian dogfish meal. The results of this study are summarized in Table 3. The method using potassium persulfate gave slightly higher results in nearly all cases, but the difference between the methods was usually less than 0.1 of one per cent. No nitrogen was found in the potassium persulfate. The use of this reagent did cut down the time of digestion somewhat. However, the results do not indicate that a change in the official method is necessary for the ordinary fertilizer products. With special products, such as Canadian dogfish meal, meat scraps, and coconut meals, the use of potassium persulfate might be desirable, and further work should be done with these products.

TABLE 3.—*Comparison of the total nitrogen in various organic fertilizers by the official method and by the method using potassium persulfate*

MATERIAL	OFFICIAL METHOD	POTASSIUM PERSULFATE METHOD
	per cent	*per cent*
Crab Meal	10.42	10.46
Cottonseed Meal	6 40	6.44
Castor Pomace	5.19	4.97
Process Tankage	6.73	6.82
Garbage Tankage	3.32	3.28
Chicken Manure	2.63	2.71
Sewage Sludge	0.92	0.94
Ground Fish	8.11	8.20

In *Methods of Analysis, A.O.A.C.,* 1935, par. **19(g)**, p. 23, an error has been noted in the preparation of the sodium hydroxide solution. It reads as follows: "dissolve approximately 450 g of commerical NaOH, free from nitrates, in 1 liter of water. This solution should have a sp. gr. of 1.43–1.48." A solution containing 450 grams of sodium hydroxide per liter would have a sp. gr. of only 1.36. Although considerable latitude should be allowed in the concentration of this solution, it would be well to modify the last sentence of this paragraph to read: "A solution having a sp. gr. of 1.36 or higher may be used."

RECOMMENDATIONS[1]

It is recommended—

(1) That a collaborative study be made of the beaker method in comparison with other methods for the determination of water-insoluble nitrogen.

(2) That the use of potassium persulfate along with mercury as catalyst in the determination of total nitrogen be studied on such materials as Canadian fish meal, meat scraps, and coconut meals.

(3) That the last sentence in par. 19(g), p. 23, of the 1935 edition of *Methods of Analysis,* be deleted, and the following sentence be incorporated in its place: "A solution having a sp. gr. of 1.36 or higher may be used."

(4) That the reduced iron method for the determination of nitrate nitrogen in mixed fertilizers or nitrate salts described under Sec. 31, p. 26, of the 1935 edition of *Methods of Analysis,* be deleted (final action).

REPORT ON MAGNESIUM AND MANGANESE IN FERTILIZERS*

By John B. Smith, *Associate Referee,* and E. J. Deszyck (Agricultural Experiment Station, Kingston, R. I.)

The study of methods for determining magnesium and manganese, *This Journal,* 20, 252 (1937); 21, 277 (1938), was continued. As in previous years, the writers are indebted to many collaborators for ideas, criticism, and analytical assistance.

MAGNESIA

Progress made during the year is reported under the topics outlined in previous reports. The emphasis has been on methods for acid-soluble magnesia shorter than the official method.

Collaborative Analyses

Seven methods were sent to collaborators, five for magnesia and two for manganese. As the same collaborators undertook work on both elements, description of the samples and presentation of results are combined in this section.

The procedures are as follows:

1. Acid-soluble Magnesia, Official. *Methods of Analysis,* A.O.A.C., 1935, p. 34, 54, as modified in 1936, *This Journal,* 20, 252 (1937).

2. The Bartlett-Tobey Method developed at the Maine Agricultural Experiment Station and modified in minor details by the writers. The detailed procedure follows:

Weigh 2.5 g. of fertilizer into a 250 cc. volumetric flask, add 30 cc. of HNO_3 and 10 cc. of HCl, and boil for 30 minutes. Cool, make to volume, mix, filter thru a dry filter paper, and transfer a 100 cc aliquot to a 400 cc. beaker. Add a few drops of methyl red. Add NH_4OH until the solution is yellow, then HCl until barely pink. Add 15 cc. of a saturated solution of NH_4 oxalate, adjust the solution to pH 5.0 (a faint pink color) by the addition of HCl (1+4), or NH_4OH (1+4), boil for a few minutes, cool, and again adjust the reaction to pH 5.0, adding more methyl red if

necessary. Stir thoroughly and allow the solution to stand until the precipitate settles. Filter through a 11 cm. filter paper fine enough to retain Ca oxalate and wash 10 times with hot water. To the filtrate add 2 cc. of 10% HCl, evaporate to a volume of approximately 100 cc., and add 5 cc. of a 10% Na citrate solution and enough concentrated NH_4OH to make the solution alkaline. (Blue with bromothymol blue). If the fertilizer does not contain soluble phosphoric acid, add 5 cc. of a 10% solution of $(NH_4)_2 HPO_4$. Stir vigorously until precipitation is completed. Add 15 cc. of concentrated NH_4OH and allow to stand at least 2 hours, stirring frequently, or allow to stand overnight. Transfer the precipitate to a small filter or filtering crucible. Wash, and ignite as directed under II, 54. If $Mn_2P_2O_7$ is present, correct for it as directed under II, 54.

3. A volumetric modification of the official method suggested by P. McG. Shuey. Briefly, this includes solution of a 4 gram sample in a Kjeldahl flask with HNO_3, H_2SO_4, and KNO_3, treatment of the entire sample with alcohol and water to precipitate $CaSO_4$, a single precipitation of $MgNH_4PO_4$, washing free from ammonia with 50% alcohol and titration of the precipitate with 0.1 N HCl and 0.1 N NaOH.

4. Magnesia insoluble in 4% citric acid titrated to pH4, with NH_4OH 90 minutes, 90°–95° C.

5. Water-soluble magnesia in magnesium sulfate and sulfate of potash-magnesia.

6. A volumetric periodate method for acid-soluble manganese, *This Journal*, 21, 292 (1938).

7. A colorimetric modification of Method 6, suggested by H. D. Haskins and J. W. Kuzmeski of the Massachusetts Agricultural Experiment Station.

Collaborators submitting results were: C. A. Butt and C. M. Cartledge, International Agricultural Corporation; E. J. Deszyck, Rhode Island Agricultural Experiment Station; W. Y. Gary, Florida Agriculture Department; E. T. Hord, North Carolina Department of Agriculture; L. F. Rader, Jr., U. S. Department of Agriculture; P. McG. Shuey, Shuey and Co.; Oscar I. Struve, Eastern States Cooperative Milling Corporation. The amount of work requested was greater than usual, and full acknowledgment is made of the generous help received.

The samples are described in Table 1. The first is high in phosphate; the second contains considerable organic matter; and the third contains only inorganic materials including silica in sand. The results are shown in Tables 1 and 2.

Acid-Soluble Magnesia

Hoffman Method.—This method, now official, has received favorable comment for accuracy, but has been criticized by many for its length. One of the collaborators states the criticism very clearly in this paragraph: "From our standpoint, the time saved by the short-cut magnesia methods overshadows in importance the slight difference in the results obtained. It has been inconvenient, to say the least, to have to wait the larger portion of a week before magnesia tests could be completed, while fertilizer analyses were otherwise complete within a day or two after receipt of samples. For the present my sentiment is that the Hoffman method should be retained as the official method; but, if at all possible,

TABLE 1.—*Samples for collaborative analysis*

SAMPLE	INGREDIENTS	POUNDS	ADDED MgO (PER CENT)			
			KIESERITE	DOLOMITIC LIMESTONE	NON-CARRIER	TOTAL
1. 8-16-12	Sulfate of ammonia	360				
	Urea	120				
	Nitrate of potash	200				
	Tankage	140				
	Triple superphosphate	710				
	Muriate of potash	300				
	Dolomitic limestone	100				
	Kieserite	70	1.01	0.95	0.44	2.40
2. 5-8-7	Sulfate of ammonia	170				
	Urea	50				
	Tankage	515				
	Superphosphate	720				
	Muriate of potash	275				
	Dolomitic limestone	200				
	Kieserite	70	1.01	1.89	0.22	3.12
3. 7-6-6	Sulfate of ammonia	570				
	Nitrate of soda	120				
	Superphosphate	540				
	Muriate of potash	236				
	Dolomitic limestone	200				
	Kieserite	100				
	Quartz sand, 40-mesh	234	1.44	1.89	0.11	3.44

4. Kieserite
5. Sulfate of potash magnesia   Mn
6.* Sample 1, but substitute 125 lbs. manganese sulfate, 24.94 per cent   *per cent*
   Mn, for 70 lbs. Kieserite, 55 lbs. dolomitic limestone   1.56
7.* Sample 2, but substitute 85 lbs. manganese sulfate for 85 lbs. dolo-
   mitic limestone   1.06
8.* Sample 3, but substitute 43 lbs. manganese sulfate for 43 lbs. sand   0.54

* The ingredients, other than manganese sulfate, supplied traces of manganese too small in amount to affect results for these samples.

work should be continued on the short-cut methods with a view to ar-
riving at a procedure giving reasonably satisfactory results in materially
less time, as an alternative procedure." The Associate Referee is in full
accord with the idea, and believes that the objective can be accomplished
without sacrifice of accuracy. The Hoffman method, as stated in previous
reports, has an authoritative background, and has been advocated and
adopted because it assures accuracy. Thus, it fills the need for a reference
method, with which to compare other procedures, but justifiable adverse
criticism of the method for routine work has been anticipated.

TABLE 2.—*Collaborators' results for MgO and Mn (per cent)*

	SAMPLES					
	1	2	3	1	2	3
COLLABORATORS	ACID-SOLUBLE MgO (PER CENT)					
	METHOD 1, OFFICIAL			METHOD 2, BARTLETT-TOBEY		
Butt, Cartledge	2.19	2.92	3.20	2.40	3.07	3.29
Deszyck	2.47	3.25	3.56	2.37	3.17	3.47
Gary	2.21	3.01	3.37	2.58	3.26	3.57
Hord	2.22	3.00	3.38	2.38	3.20	3.46
Rader	2.48	3.24	3.42	2.44	3.24	3.42
Shuey	—	—	—	2.41	3.26	3.39
Struve	2.32	3.00	3.37	2.48	3.28	3.41
Average	2.31	3.07	3.38	2.44	3.21	3.43
Recovery of MgO (%)	96	98	98	102	103	100
	METHOD 3, SHUEY			METHOD 4, ACID-CITRATE-INSOLUBLE		
Butt, Cartledge	2.48	3.24	3.46	0.60	1.60	1.41
Deszyck	2.40	3.21	3.54	0.36	1.79	1.37
Gary	—	—	—	—	—	—
Hord	2.25	3.05	3.36	0.78	1.56	1.14
Rader	2.46	3.35	3.67	0.66	1.41	1.13
Shuey	2.42	3.22	3.52			
Struve	2.45	3.20	3.55			
Average	2.41	3.21	3.52	0.60	1.59	1.26
Recovery of MgO (%)	100	103	102	45*	22*	37*

	WATER-SOLUBLE MgO	
	KIESERITE	SULFATE OF POTASH MAGNESIA
Butt, Cartledge	28.73	—
Deszyck	28.67	10.46
Hord	28.30	10.17
Rader	29.02	10.87
Shuey	28.61	—
Struve	28.67	10.26
Average	28.67	10.44

	SAMPLES					
	4	5	6	4	5	6
	ACID-SOLUBLE Mn (PER CENT)					
	VOLUMETRIC METHOD			COLORIMETRIC METHOD		
Deszyck	1.55	1.06	0.65	1.58	1.04	0.65
Gary	1.21†	1.04	0.68			
Hord	1.72	1.12	0.63			
Rader	1.63	1.04	0.63	1.75	1.04	0.67
Shuey	1.52	1.00	0.56			
Average	1.61	1.05	0.63	1.67	1.04	0.66

* Recovery of MgO added as dolomitic limestone, as citrate-soluble MgO, subtracting 0 08, 0 12, and 0.07 % citrate-insoluble MgO from non-carriers in Samples 1, 2, and 3, respectively, from results by analysis.
† Omitted from average. Gary reported 1.58 % Mn by a bismuthate method.

Bartlett-Tobey Method.—Several alternatives have been suggested. The first was an attempt by J. M. Bartlett and E. R. Tobey* to shorten the time needed for the orthodox procedure for separating calcium and magnesium as the oxalates. The authors of the method pointed out deviations from text-book versions of the method but found no significant deviations in results. This was a promising start, and the procedure has now been tried collaboratively for three years, either for acid-soluble magnesia or for the water-soluble fraction. It has been modified in minor details in this laboratory to meet possible criticisms. Extraction with 1 per cent hydrochloric acid has been changed to treatment with nitric acid and hydrochloric acid to destroy organic matter and increase the probability of dehydrating silica, but the procedure, as published in this report, follows the original suggestion quite closely. The principal deviations from the standard procedure are single precipitations of calcium oxalate and magnesium ammonium phosphate and shorter time allowances for formation of these precipitates. The single precipitation has precedent in methods of this Association, both for calcium oxalate and magnesium ammonium phosphate (*Methods of Analysis, A.O.A.C.*, 1935, II, 9; XII, 10, 11, 12; XVII, 24 and XXVI, 19, 20), although not under conditions exactly similar to those here described. Ross, *This Journal*, 11, 180 (1928), after thorough study of the precipitation of magnesium ammonium phosphate for the determination of phosphoric acid in fertilizers decided that a double precipitation in a routine method did not add sufficient accuracy to justify the increased time required.

Comparisons of the Bartlett-Tobey modification with the official method may be found in the two previous reports on this topic, cited previously, and in Table 2. In addition, 37 samples of commercial brands of fertilizer that had been analyzed at the Maine Agricultural Experiment Station and 33 samples from the North Carolina Department of Agriculture were supplied by the chemists of those institutions and analyzed at this Station. The results are summarized in Table 3. On the average the Bartlett-Tobey method gave values 0.05 per cent greater than those by the official method. Results by collaborators have been equally consistent by both methods. Average differences between two laboratories for individual samples by the official method have been about equal to the differences between the two methods at one laboratory. These differences are somewhat greater where the official method at one laboratory is compared with the Bartlett-Tobey method at another. From the results at hand, either method seems sufficiently accurate for control work, with the shorter method giving slightly higher results. This tendency does not appear to be correlated with the percentages of magnesia in the mixture or with the percentages of calcium oxide actually determined in the 70 fertilizers mentioned. Reprecipitation of calcium oxalate and magnesium

* Private communication.

TABLE 3.—*Summary of comparisons of the official method with the Bartlett-Tobey procedure*

		MgO per cent
12	Collaborative samples	
	Average by Bartlett-Tobey Method greater	0.05
	Mean deviation, Official Method	0.09
	Mean deviation, Bartlett-Tobey Method	0.08
37	Samples. Analysis at Maine Agr. Exp. Station and the Rhode Island Exp. Station	
	Average of differences for individual samples	
	Official Method, Maine vs. Rhode Island	0.08
	Official and Bartlett-Tobey Methods,	
	Maine vs. Rhode Island	0.13
	Rhode Island alone	0.09
	Average, all analyses, Bartlett-Tobey greater	0.04
33	Samples analyzed at North Carolina Department of Agriculture and Rhode Island Exp. Station	
	Average of differences for individual samples	
	Official Method, N.C. vs. R.I.	0.08
	Official and Bartlett-Tobey Methods	
	N.C. vs. R.I.	0.11
	Rhode Island alone	0.07
	Average, all analyses, Bartlett-Tobey greater	0.05

ammonium phosphate did not change the results by the Bartlett-Tobey method consistently at this laboratory. Hord, however, with results by the Bartlett-Tobey method in excellent agreement with the averages for those of other collaborators this year, reduced these results consistently by reprecipitation of the magnesium ammonium phosphate. His results were then in better agreement with averages for the official method than for the shorter procedure. Probably the higher results by the Bartlett-Tobey method are caused by mixtures of phosphates in the precipitate, including calcium, or by silica occlusions, and can be corrected by reprecipitation, but the added accuracy does not justify the loss of time. The magnitude of the changes that would result are of about the same magnitude as the variations from other apparently unavoidable errors.

Because of experience with the Bartlett-Tobey method, and confidence that it will fill the immediate need for a shorter method in laboratories where the number of magnesia determinations is increasing rapidly, the Associate Referee is recommending this method as a tentative method. This is done without prejudice to the other methods to be discussed below, but only because these other methods have not yet received thorough trial.

Shuey Volumetric Method.—P. McG. Shuey has suggested* a very

* Private communication.

promising adaptation of a modification of the official method combined with the acidimetric titration of magnesium ammonium phosphate discussed by Handy.[1] Briefly, the procedure includes solution and oxidation of a 4 gram sample in a Kjeldahl flask with sulfuric and nitric acids, and finishing with potassium nitrate if organic matter remains. The solution is transferred to a 200 cc. volumetric flask with 50 cc. of water, and calcium sulfate is precipitated during a 2 hour period by adding alcohol. More alcohol is added to complete the volume. The alcohol is evaporated from a filtered aliquot, and magnesium ammonium phosphate is precipitated in ammoniacal solution containing the citrate ion by stirring or standing. The precipitate is filtered on a Gooch pad, washed with alcohol (equal volumes of 95% alcohol and water), dissolved in 0.1 N hydrochloric acid, and the excess acid is titrated to the usual methyl orange color change.

This is the most rapid method that has been tried this year. The results (Table 2) are more consistent than those for the official method, and equal to those with the Bartlett-Tobey procedure. The method is decidedly promising, but needs more thorough trial next year to justify a decision concerning its ultimate usefulness. As with any single precipitation of magnesium ammonium phosphate, several types of contamination are possible. Some have greater effect on an ignition method, and others in titration. Several modifications are possible. The precipitate may be ignited as for other methods. The excess ammonia in the precipitate and filter may be driven off at 40° C. or by standing at room temperature. This volumetric modification may be used with the oxalate separation of calcium, allowing for simple determination of that element in the same aliquot. A great advantage of the volumetric method is the time saved in transfer of the final precipitate to a filter. Washing may be by decantation and the policing of the particles adhering to flask or beaker is avoided. The Associate Referee will gladly send copies of the detailed procedure to any who apply.

A third method is the application of the volumetric modification of the 8-hyroxy-quinoline procedure recommended after trial by W. B. Byers. This method is said to be very rapid and to allow for the complete separation of manganese, thus avoiding the correction for this element in the final precipitate. W. J. Gascoyne, after experience with a considerable number of samples, suggested a method somewhat like the Bartlettt-Tobey procedure but which separates calcium as the oxalate and phosphate at about the neutral point, in the presence of ammonium chloride, rather than adjusting carefully at a lower pH to avoid precipitation of calcium phosphate. Each of these suggestions deserves more careful attention than has been possible this year.

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[1] *J. Am. Chem. Soc.*, **22**, 31 (1900).

Active Magnesia

Previous reports have discussed in detail this fraction, which is intended to measure the magnesia available to plants in a single cropping season. This year real progress was made in determining the average rate of decomposition of dolomite in the soil. Part of the work was a collaborative effort organized by the Sub-Committee on Fertilizer Reaction from the American Society of Agronomy, *This Journal*, 22, 137, 142 (1939). Independent studies were made at the Indiana Experiment Station. The fertilizers and dolomites used were made available to the Associate Referee for chemical studies to find a solvent that will correlate with the reactivity of the soil. Results from the pot tests were not ready in time for a satisfactory completion of the laboratory work but preliminary tests with the neutral ammonium citrate solution used for phosphoric acid and the pH 4.0 ammonium citrate solution, which has shown greater promise, *This Journal*, 21, 277 (1938), were tried in this laboratory. The acid citrate solution was also used by the collaborators for the samples reported in Table 2. The results (Table 4) show definitely that neutral ammonium citrate does not dissolve enough of the dolomites for the purpose. The acid citrate, although far from perfect shows a considerable degree of correlation, and is the most promising solvent tried. As presented to the collaborators, however, it is unsatisfactory, for it does not give concurring results. It must be rewritten to employ lower and accurately controlled temperatures, preferably 65°, to agree with the method for citrate-insoluble phosphoric acid and a shorter heating period, and to allow for the determination of the magnesia dissolved, rather than the insoluble portion. This can doubtless be done by varying pH and citrate concentration, and will be attempted next year.

If the solvent is to prove useful, it should apply to the dolomites alone as well as in mixtures. Otherwise it will be difficult to calculate fertilizer formulas to contain the desired amounts of active magnesia. J. W. Kuzmeski has supplied important information on this topic, *This Journal*, 22, 147 (1939). Treating 0.2 gram charges of nine dolomitic limestones as directed for the acid-citrate solution at pH 4.0 he finds considerable variations among different limestones of equal fineness and among different separates of the same limestone. Average percentage solubilities for magnesia were as follows: 60–80 mesh, 57; 80–100 mesh, 66; 100–200 mesh, 84; through 200 mesh 98.6; mill-run, 71. These results are similar in general to the solubility of the dolomites reported in Table 4.

Water-Soluble Magnesia

Laboratory studies of this fraction have been held in abeyance this year, for the problem is agronomic rather than chemical. An analytical procedure that was tried has worked with apparent satisfaction in Maine for several years. Agronomists, however, are not agreed on the necessity

TABLE 4.—*Average decomposition of dolomites in fertilizer mixtures in eight acid soils and the average solubility of these dolomite fertilizer mixtures in two citrate solutions*

	RECOVERY OF MgO		
MIXTURE	DECOMPOSITION BASED ON RESIDUAL CARBONATES IN THE SOIL	NEUTRAL NH₄-CITRATE 1 G. CHARGE, 1 HR., 65°C.	4% CITRATE MADE TO pH 4.0 WITH NH₄OH, 1 G. CHARGE, 90 MIN., 90–95°C.
	per cent	*per cent*	*per cent*
Dolomite A			
Mesh			
20– 40	24	2	43
40– 60	41	7	57
60– 80	55	7	57
80–100	62	10	60
100–200	75	10	74
Through 200	85	25	83
Composite	57	6	61
Dolomite B, Composite	63	9	70
Dolomite C, Composite	51	3	38

The fertilizers, dolomites, and pot tests are described by Emerson R. Collins and Paul R. Dawson in papers presented before this Association, *This Journal*, 22 137, 142 (1939). The average decomposition of each dolomite used was calculated from a preliminary report made available to the Associate Referee.

for a separate determination of this portion. Many believe that a determination of active magnesia will suffice; others stress the importance of water-soluble magnesia for tobacco in the South East, potatoes in Maine, and for acute deficiencies elsewhere. Because of this uncertainty, it seems best to await a more definite demand before recommending official status for the method for water-soluble magnesia.

Water-Soluble Magnesia in Magnesium Sulfate, Kieserite, and Sulfate of Potash Magnesia

This topic is distinct from that discussed above. These materials are marketed on the basis of water-soluble magnesia content and a uniform method is necessary, but it is not intended for use with fertilizer mixtures. The procedure recommended last year, *This Journal*, 21, 77 (1938), was tried by six collaborators, and the results (Table 2) show sufficient agreement to justify the procedure. As was true last year, there is a rather wide variation between the extremes but a reassuring tendency for a good proportion of the results to group about the means. The greatest divergence is for the sample of sulfate of potash magnesia this year.

Doubtless this method can be combined later with the method ultimately chosen as most rapid and satisfactory for measuring magnesia in

acid solution. After solution is accomplished the conditions are much the same, whether the solvent is acid or water.

ACID-SOLUBLE MANGANESE

The volumetric periodate method, published last year, *This Journal*, 21, 292 (1938), was tried by the collaborators. As stated last year, it was recommended by F. B. Carpenter and published originally by Willard and Thompson.[1] It should be applicable in the absence of chromium, cobalt, and cerium, with the precaution of removal of chlorides. The collaborative results (Table 2) show satisfactory agreement. W. Y. Gary reported a low result for Sample 1, but found the proper amount of manganese by the older bismuthate method, with which he was more familiar and which he prefers. The reason for this difficulty does not appear, and it was not experienced by the other analysts, nor by Gary for the samples with less manganese. The procedure seems to justify recommendation as a tentative method, and it may be modified later if experience shows this to be necessary.

One detail must be added to the description of the method as published last year. Preparation of the standard ferrous sulfate solution should read: "0.091 N. 25.3 grams of $FeSO_4 \cdot 7H_2O$ and 25 cc. of H_2SO_4 in 1 liter of solution. Standardize with the 0.0910 N $KMnO_4$." The sulfuric acid is necessary to stabilize the solution.

Colorimetric Periodate Method.—The volumetric method is generally applicable to all amounts of manganese in fertilizers and manganese carriers used as ingredients, and is necessary on that account. For lower percentages, such as usually occur in mixed fertilizers, it is probable that a colorimetric modification[2] is shorter and can be used to advantage.

As in many other instances, the Associate Referee is indebted to H. D. Haskins and J. W. Kuzmeski for independent work made available for his use. Space does not allow a complete account of the work, but the most pertinent details may be described briefly. Using the samples distributed last year, Nos. 3, 4, 5 in the last previous report, they tried several methods of solution. The one finally preferred is digestion of a 1 gram sample in a 200 cc. volumetric flask with 10 cc. of sulfuric acid and 30 cc. of nitric acid at the boilding point, and evaporation until white fumes appear. Five cc. of 85 per cent phosphoric acid is added with 30 cc. of water. The solution is brought to a boil and filtered. The filter is washed with water, 0.3 of potassium periodate is added for each 15 mg. of manganese, and the mixture is heated with stirring for 30 minutes. After cooling, the solution is diluted and made to a convenient, measured volume for comparison with standard potassium permanganate in a colorimeter. By this method, Kuzmeski reports 1.02, 1.02, and 1.01 per cent of manganese for Samples 3, 4, and 5, respectively. By the volumetric

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[1] *Ind. Eng. Chem. Anal. Ed.*, 3, 399 (1931).
[2] *J. Am. Chem. Soc.*, 39, 2366 (1917).

method, two analysts at the Rhode Island Experiment Station find 1.03, 1.00 and 1.02 per cent manganese for these same samples.

The method was tested further and gave satisfactory results in this laboratory. As written for the collaborators, however, it was not entirely successful in the hands of the few who tried it. The results, reported in Table 2, show excellent agreement by two analysts, and especially good for Samples 3 and 4. Hord, however, reports an unsuccessful attempt. The principles of the procedure are sound, and it is only necessary to rewrite the method for more definite control of conditions to make it a valuable, modification of the volumetric method. This will be attempted next year.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for the determination of magnesia in water-soluble compounds, *This Journal*, 21, 77 (1938), adopted as a tentative method last year, be adopted as official (first action), and that it be entitled, "Magnesia in Water-soluble Compounds Applicable to Sulfate of Potash Magnesia, Sulfate of Magnesia, and Kieserite."

(2) That the Bartlett-Tobey method for acid-soluble magnesia be adopted as a tentative method.

(3) That the Shuey volumetric method and other modifications of the present official method for acid-soluble magnesia be studied.

(4) That the study of methods for active magnesia in mixed fertilizers be continued.

(5) That the volumetric method for acid-soluble manganese in fertilizers and manganese salts, published in *This Journal*, 21, 292 (1938), but with minor changes noted in this report, be adopted as a tentative method.

(6) That the colorimetric modification for acid-soluble manganese discussed in this report be further studied.

The paper, entitled "Effect of Particle Size on the Solubility of Magnesium in Dolomite and Magnesic Limestone in 4 per cent Citric Acid Solution Adjusted to *p*H 4.0 with Ammonium Hydroxide," by J. W. Kuzmeski, was published in *This Journal*, 22, 147 (1939).

REPORT ON POTASH

By O. W. FORD (Purdue University Agricultural Experiment Station, West Lafayette, Ind.), *Associate Referee*

No collaborative work was carried out this year. At the 1937 meeting the General Referee on Fertilizers recommended: "That the Associate

Referee on Potash be requested to ascertain whether any other state besides California forbids the use of the present official method for potash on account of provisions of the laws regarding water-soluble potash and to make any recommendations regarding this matter that seem advisable."

The following questionnaire was sent to the control officials of the 38 states having fertilizer control laws:

1. The Fertilizer Control Law of the State of ———————————— (permits, does not permit) the use of the present official method for potash.
2. The State of ———————————— (uses, does not use) the present official method for potash in fertilizer control work.
3. Do you regard the present official method for potash in the State of ————— as satisfactory (yes, no)?
4. Present-day fertilizers in the State of ———————————— (produce, do not produce) determinable amounts of water-insoluble residue, which will be weighed up as K_2PtCl_6 unless removed by filtration previous to the precipitation with H_2PtCl_6.
5. Please insert below any comment on the method, whether critical or otherwise.

A summary of the replies to Questions 1 and 2 of the questionnaire appears in Table 1.

TABLE 1.—*Use of the official method*

NUMBER OF STATES REQUIRING USE OF OFFICIAL METHOD	NUMBER OF STATES PERMITTING THE USE OF OFFICIAL METHOD	NUMBER OF STATES NOT PERMITTED TO USE OFFICIAL METHOD	NUMBER OF STATES USING OFFICIAL METHOD	NUMBER OF STATES NOT USING OFFICIAL METHOD
4	31	3	34	4*

* Two states are using either the perchloric acid or a modified perchloric acid method for potash.

A summary of the replies to Questions 3 and 4 of the questionnaire is given in Table 2.

TABLE 2.—*Comments on the official method*

NUMBER OF STATES REPORTING METHOD SATISFACTORY	NUMBER OF STATES REPORTING METHOD UNSATISFACTORY	NUMBER OF STATES NOT REPORTING WATER-INSOLUBLE RESIDUES	NUMBER OF STATES REPORTING WATER-INSOLUBLE RESIDUES	NUMBER OF STATES REPORTING CORRECTIONS FOR WATER-INSOLUBLE RESIDUES
23	6	23	12	10

OTHER COMMENTS ON THE OFFICIAL METHOD

One state reported that residues were found equivalent to potassium oxide ranging from 0.1 to 0.15 per cent when platinum dishes were used. It was necessary to filter before precipitating with potassium chloroplatinate to obtain satisfactory results.

Commercial chemists have reported water-insoluble residues equivalent to 0.23 per cent potassium oxide, and many report that they are

making corrections for these residues by dissolving out the potassium chloroplatinate and weighing back.

Allen and Gault, *This Journal*, 20, 101 (1937), state that they found more water-insoluble residue with the present official method than with the former method and that the residue increased with the increase of organic matter in the fertilizer. The residue was not affected by re-washing with the reagents used in the method.

More than a year ago, following the installation of natural gas in this laboratory but before final adjustments were made, water-insoluble residues were encountered that were larger than those reported by Kray-bill and Thornton, *This Journal*, 18, 269 (1935). Their work, however, was completed prior to the installation of the natural gas. Since there was available no accurate means of measuring the temperature of ig-nition of the gas during the final burning off of the potash it was thought advisable to determine the effect on the amount of water-insoluble resi-dues of ignition at different controlled temperatures in a muffle furnace.

EFFECT OF IGNITION TEMPERATURE ON
WATER-INSOLUBLE RESIDUES

For this work, inspection sample L-4949, a 1–11–3 fertilizer with a high content of organic matter, was selected. A composite solution re-sulting from twelve 2.5 gram weighings from a portion of this sample ground to pass a 0.5 mm. sieve was used for all the determinations listed in Table 3. The water-insoluble residues were obtained as increased weight on a tared sintered glass filter after the potassium chloroplatinate had been filtered through it, weighed, and dissolved out and reweighed.

By using a sintered glass filter losses of weight occasioned when an asbestos pad is used were avoided, and many weighings and leachings out of the potassium chloroplatinate could be made before the filter would clog enough to slow up the filtration.

When the filter becomes clogged it can be cleared readily by treating it with aqua regia. With a filter of medium porosity (like a Jena BG-3), the speed of filtration is equal to that of a Gooch padded with asbestos. The one advantage of the sintered glass filter is that no increase of weight is obtained in a determination after the potassium chloroplatinate has been dissolved out unless water-insoluble residue is encountered. From the standpoint of additional work required this method of removing the residue is preferable to filtering before precipitation with potassium chloroplatinate.

Ohio analysts reported that they are removing the water-insoluble residue by filtration through an A. H. Thomas 16 G3 sintered glass filter.

It will be observed that at 550° C. noticeable residues were obtained and that at 650° and 750° correspondingly less residues were obtained, although one or two in answering the questionnaire indicated that they

TABLE 3.—*Effect of ignition temperature on water-insoluble residues in the determination of potash by the official method*
(Results expressed in percentage.)

SAMPLE NUMBER	AT 550°C.				AT 650°C.				AT 750°C.			
	K_2O	DIFFERENCE ± FROM AVERAGE	K_2O CALCULATED FROM RESIDUE	DIFFERENCE ± FROM AVERAGE	K_2O	DIFFERENCE ± FROM AVERAGE	K_2O CALCULATED FROM RESIDUE	DIFFERENCE ± FROM AVERAGE	K_2O	DIFFERENCE ± FROM AVERAGE	K_2O CALCULATED FROM RESIDUE	DIFFERENCE ± FROM AVERAGE
4949	3.22	−0.06	0.12	−0.17	3.02	−0.03	0.08	−0.01	2.95	+0.14	0.03	−0.01
4949	3.39	+0.11	0.30	+0.01	2.99	−0.06	0.03	−0.06	2.71	−0.14	0.02	−0.02
4949	3.22	−0.06	0.39	+0.10	3.10	+0.05	0.08	−0.01	2.95	+0.14	0.04	—
4949	3.45	+0.17	0.35	+0.14	3.08	+0.03	0.06	−0.03	3.02	+0.19	0.03	−0.01
4949	3.33	+0.05	0.25	−0.04	3.10	+0.05	0.08	−0.01	3.06	+0.25	0.08	+0.04
4949	3.31	+0.03	0.21	−0.08	3.02	−0.03	0.08	−0.01	2.96	+0.15	0.00	−0.04
4949	3.18	−0.10	0.28	−0.01	2.99	−0.06	0.08	−0.01	2.91	+0.10	0.00	−0.04
4949	3.20	−0.08	0.31	+0.02	3.10	+0.05	0.17	+0.08	2.98	+0.17	0.06	+0.02
4949	3.22	−0.06	0.30	+0.01	2.99	−0.05	0.08	−0.01	2.63	−0.18	0.04	—
4949	3.37	+0.09	0.30	+0.01	3.05	—	0.16	+0.07	3.01	+0.20	0.08	+0.04
4949	3.20	−0.08	0.24	−0.05	3.03	−0.02	0.08	−0.01	2.69	−0.12	0.01	−0.03
4949	3.25	−0.03	0.39	+0.10	3.07	+0.02	0.09	—	2.89	+0.08	0.11	+0.07
High	3.45	+0.17	0.39	+0.10	3.10	+0.05	0.17	+0.08	3.06	+0.25	0.11	+0.07
Low	3.18	−0.10	0.12	−0.17	2.99	−0.06	0.03	−0.06	2.63	−0.18	0.00	−0.04
Average	3.28		0.29		3.05		0.09		2.81		0.04	
Corrected for Residue	2.99				2.96				2.77			

would recommend heating above 750° C. to eliminate residue. It is possible that heating at 750° C. for a long period will volatilize some of the potash and give lower results. At least with the sample taken in this case the lowest results were obtained when the ignition was done at 750° C. It is recognized that any ignition carried out in an electric muffle will take longer and the dishes will be heated longer at the highest temperature of the ignition than will be the case when the ignition is finished off over a Meeker burner, even though the temperature of the Meeker may be as high as that of the muffle. Thus the muffle would afford a chance for volatilization of potash if any volatilizes at that temperature.

From Table 3 it will be seen that the most concordant results were obtained by ignition at 650°C. At this temperature the residues were not significant. In case the conditions of ignition cannot be sufficiently controlled to avoid residues the weight of the potassium chloroplatinate should be determined by weighing, dissolving out with water, and re-weighing.

EFFECT OF FINENESS OF GRINDING ON UNIFORMITY OF RESULTS

Studies were also made relative to errors resulting from non-uniformity of the 2.5 gram samples weighed out for the official potash determination. One laboratory reported in the questionnaire that the potash salts were ground to pass through a 0.5 mm. sieve. Others have indicated at various times that that was the procedure they followed when they could not obtain good checks.

On several occasions during the past year the Associate Referee had difficulty in obtaining concordant results. On one occasion it was necessary to grind a portion of the reserve sample to pass a 0.5 mm. sieve. Two samples that gave exceptionally variable results were picked for investigation. From the reserve bottles of these two samples three 2-ounce portions were taken and ground to pass the 2-, 1-, and 0.5-mm. sieves, respectively. From each sample twelve 2.5 gram samples were weighed out for the determination of potash.

Table 4 lists the results obtained on inspection sample L-6289 (0-20-20), with ground tobacco as a conditioner, while Table 5 lists the results obtained on inspection sample L-5883, a muriate of potash (0-0-50) that had been cut with sand.

All bottles of fertilizer were full at the start, and all 12 samples weighed for determination were drawn from the bottles in the order listed in Tables 4 and 5.

The surprising thing about the results in Table 4 is that the coarsest ground portion produced both the highest and lowest potash values as well as the most erratic set of results, showing how slight is the chance to obtain a correct value on a product ground to pass only the 2-mm. sieve. As the degree of fineness was increased, more concordant results

TABLE 4.—*Effect of fineness of grinding on the uniformity of potash determinations*

SAMPLE NUMBER	2 MM.	DIFFERENCE ± FROM AVERAGE	1 MM.	DIFFERENCE ± FROM AVERAGE	0.5 MM.	DIFFERENCE ± FROM AVERAGE
		per cent		*per cent*		*per cent*
6289	21.16	+1.54	20.78	+0.62	19.76	−0.11
6289	20.70	+1.08	20.50	+0.34	19.72	−0.15
6289	19.99	+0.37	20.40	+0.24	19.70	−0.17
6289	20.70	+1.08	20.28	+0.12	19.62	−0.25
6289	19.71	+0.09	20.50	+0.34	19.74	−0.13
6289	19.96	+0.34	20.74	+0.58	20.08	+0.21
6289	20.24	+0.62	19.50	−0.66	20.30	+0.43
6289	19.62	—	20.25	+0.09	19.87	—
6289	18.06	−1.56	19.61	−0.55	19.44	−0.43
6289	17.88	−1.74	20.31	+0.15	19.84	−0.03
6289	16.86	−2.76	19.21	−0.95	20.24	+0.37
6289	20.54	+0.92	19.92	−0.24	20.08	+0.21
Average	19.62	—	20.16	—	19.87	—
Low	16.86	−2.76	19.21	−0.95	19.44	−0.43
High	21.16	+1.54	20.78	+0.62	20.30	+0.43
Difference between High and Low	4.30		1.57		0.86	
5883	49.18	+0.79	49.07	+1.64	47.89	—
5883	48.72	+0.33	48.49	+1.06	48.06	+0.17
5883	49.34	+0.95	48.45	+1.02	48.10	+0.21
5883	48.25	−0.14	48.53	+1.10	47.73	−0.16
5883	49.02	+0.63	48.58	+1.15	47.82	−0.07
5883	48.57	+0.18	48.30	+0.87	47.87	−0.02
5883	48.22	−0.17	48.53	+1.10	48.06	+0.17
5883	48.26	−0.13	48.29	+0.86	48.21	+0.32
5883	48.64	+0.25	47.27	−0.16	47.56	−0.33
5883	47.38	−1.01	48.65	+1.22	47.75	−0.14
5883	47.68	−0.71	48.61	+1.18	48.14	+0.25
5883	47.48	−0.89	48.39	+1.04	47.91	+0.02
Average	48.39	—	47.43	—	47.89	—
Low	47.38	−1.01	47.27	−0.16	47.56	−0.33
High	49.34	+0.95	49.07	+1.64	48.21	+0.32
Difference between High and Low	1.96		1.80		0.65	

were obtained. If these relationships hold for other types of samples it would be advisable to change the official method to permit grinding to pass a sieve finer than the 1 mm. now permitted, especially on those samples that did not give concordant results.

METHODS OF PLATINUM RECOVERY

Investigation of the methods of platinum recovery submitted by various control and commercial chemists resulted in the selection of four

for further investigation. Two of these specify zinc in the reduction and two specify aluminum. The methods and the remarks of their sponsors follow:

METHOD 1

A. Pt from Alcoholic Washings

(1) *Recovery and Reduction.*—Continually evaporate the washings in a fairly large porcelain dish on a steam bath, and keep rinsed down between additions with distilled water. (Evaporation of the alcohol completes the reduction. A filter paper added to the dish prevents excessive sticking of the Pt on the sides of the dish.)

(2) *Purification.*—Treat the residue in dish from (1) with 3 portions of HCl (1 + 3), or until traces of iron no longer appear, transfer to a small porcelain dish, and heat in a muffle at 650° C. Cool, extract with 2 portions of HNO_3 (1+4), wash, dry, and weigh.

(3) *Preparation of Solution.*—Dissolve the Pt from (2) in a porcelain dish on a steam bath with aqua regia (3 parts HCl to 1 part of HNO_3). After solution continue evaporation with additions of HCl three times for removal of excess HNO_3, then with additions of distilled water for three times for removal of excess HCl, but do not evaporate below $\frac{1}{4}$ volume. Filter the solution and make up to 1 gram of Pt for each 10 cc. of solution, from which dilutions can be made for fertilizers of low potash content (a 10 cc. portion is normally evaporated and tested for material insoluble in 80% alcohol). In case of blank on this portion reduce the solution at this point and prepare as directed in B.

B. K_2PtCl_6 Salt Residue

(1) *Reduction.*—Dissolve all K_2PtCl_6 residues in water, filter off, and reduce with HCl and sheet Al, checking traces of final reduction with HCl and KI (a 25 cc. portion of the clear supernatant liquid is acidified with HCl and KI, and if all Pt is reduced no red color appears and vice versa).

(2) *Purification.*—Destroy final traces of Al with excess HCl. Allow the Pt black to settle, decant off the supernatant liquid several times, and finally filter on a Whatman No. 2 in a Büchner funnel and wash several times with distilled water until a negative chloride test is obtained with $AgNO_3$. Transfer the Pt black to a small procelain dish, ash in a muffle at 650° C., cool, purify further by alternate extractions with HNO_3, H_2O, and HCl, and finally dry and weigh for solution.

(3) *Preparation of Solution.*—Proceed as directed in A(3), evaporating a 10 cc. portion and testing for material insoluble in 80% alcohol. In case of a blank at this point repeat the reduction and solution as in B.

Normal time required for preparation 4 days.

METHOD 2

Pt from Waste Solutions

Reduce the accumulated Pt waste solution with H_2 to Pt black. Generate H_2 by adding HCl and 20-mesh Zn to the waste solution. (Zn dust is frequently used as a starter.) Filter the Pt black and wash with water. After washing, boil the Pt black in HCl (1 +1) for about 1 hour to remove excess metal and soluble impurities. Filter and again wash thoroughly with water. Burn off the filter paper and volatile impurities in a furnace at 750°–800° C. Redissolve the residue in aqua regia. Place the solution over a steam bath and remove all traces of HNO_3 by several additions of HCl, reducing the volume after each addition of HCl. When the solution is free from HNO_3, remove from the steam bath and filter. Test the solution for strength and adjust to the desired value. Time required for preparation 4–5 days.

METHOD 3
Pt from Scrap and/or Pt Black

(1) *Purification.*—Dissolve in aqua regia. Remove all HNO_3 by repeated evaporations just to dryness with HCl and water, using alternately, water last.

Make about a 10% solution with water and precipitate $(NH_4)_2PtCl_6$ with NH_4Cl. Allow to stand several hours, filter and wash the precipitate thoroughly with alcohol.

Ignite the precipitate—first at a low temperature, finally for 30 minutes at a very high heat. Wash by boiling in HCl, then in water, repeating several times.

Redissolve in aqua regia, remove HNO_3, dilute to a 10% solution, and neutralize with Na_2CO_3, using litmus for the end point. Filter off the precipitate containing impurities.

Heat the filtrate to boiling and reduce Pt with $NaCHO_2$. (Caution: Add the $NACHO_2$ a pinch at a time and stir well at each addition, because there is danger of excessive foaming and resultant loss of Pt.) Complete reduction is indicated when the solution clears to a water white.

Wash the Pt by boiling in HCl, then in water, and repeating several times.

Ignite first at a low heat, then for 30 minutes at high temperature. Weigh. Rewash, boiling in HCl then in water, repeating.

Redissolve in aqua regia, remove the HNO_3 as before and dilute as specified by A.O.A.C.

Recovery:—Combine the alcohol and NH_4Cl washes with $(NH)_2PtCl_6$ from the crucibles dissolved in water and add a small quantity of Zn dust, stirring frequently until reduction is complete. Allow to settle, decant the clear solution, and boil the Pt black in HCl, then in water, and repeating. Ignite and purify.

METHOD 4
Pt from Washings

Evaporate the alcoholic washings to a small bulk. Add 75–150 cc. of HCl (depending on the amount of Pt in the solution) and a piece of stick Al (10–20 grams). Continue until all Pt is reduced.

Digest precipitates of K_2PtC_6, together with asbestos, etc., with hot water. Decant the clear solution two or three times, using small portions of hot water, and then wash on the suction until all the K_2PtC_6 is free from the asbestos, etc. (Small amounts of asbestos in the solution will not interfere, in fact will aid subsequent filtration.) After all the platinic chloride is in solution, add Al and HCl as above, running the reduction until all Pt has been reduced. This can be tested as follows:

Pipet about 25 cc. of the clear solution into a 250 cc. beaker, add a few drops of HCl and a small amount of KI (in solution). If unreduced Pt is present, the solution will turn a reddish color; HNO_3 will give approximately the same color so it should not be present.

After all the Pt has been reduced, filter the Pt black on suction, using filter paper, and wash with hot water until clear. Burn the precipitate, together with the paper, in a silica dish, breaking up all large lumps with a Pt rod. After burning thoroughly digest the black in 50–200 cc. of HCl, the amount depending on the amount of Pt black, for 15–45 minutes. Then filter and wash with hot water on the suction. Again burn the precipitate and paper and digest with HCl. Follow this procedure until the HCl digestion is colorless. Add the HNO_3 and dissolve the Pt black. Standardize as directed in the official methods of the A.O.A.C.

NOTE: It is best to keep the washings and the water solution of the K_2PtCl_6 separate during the reduction of the Pt as filtering of the black will be much better.

COMMENT

In the determination of potash by the official method of the A.O.A.C., the PtCl₄ is usually made by reducing the platinum in both the alcohol washings and the hot solution of the K₂PtCl₄ precipitates by the use of zinc. This method seems to give an impure platinum black, which cannot be cleaned properly with hydrochloric acid, and it is necessary to rewash the black with nitric acid. Method 4, specifying metallic aluminum to reduce the platinum, has been found to be satisfactory without the use of nitric acid.

RECOMMENDATIONS[1]

It is recommended—

(1) That the study of the use of a factor weight or of factor weights in the determination of potash in fertilizers be continued.

(2) That the barium chloride method for the determination of potash, *Methods of Analysis, A.O.A.C.,* 1935, 31, **45, 46,** and **47,** be deleted (final action).

(3) That a study be made of the determination of potash by "dissolving out the potassium chloroplatinate and reweighing when the filtration is made on a glass sinter or asbestos padded Gooch," in place of "by filtration after ignition and solution," when platinum or silica dishes are used.

(4) That further study be made of the need for providing additional platinum solution concentrations.

(5) That a collaborative study of the four methods submitted or of other methods for the recovery of platinum be made with a view to recommending the adoption of one or more procedures.

(6) That a collaborative study be made of some modification of the present official method to prevent foaming during the boiling of the sample.

(7) That a collaborative study be made of degree of fineness of grinding with a view to elimination of the errors resulting from the non-uniformity of the 2.5 gram samples weighed out for the official potash determination.

(8) That the studies concerned with the solvent action of acid alcohol on potassium chloroplatinate be continued.

ACKNOWLEDGMENT

The writer wishes to express his gratitude to H. R. Kraybill for his counsel in connection with this report.

The paper, entitled "Filtering before Addition of Platinic Chloride in the Analysis of Fertilizers for Potash," by H. R. Allen, was published in *This Journal,* 22, 162 (1939).

[1] For report of Subcommittee A and action by the Association, see *This Journal,* 22, 51 (1939).

REPORT ON ACID- AND BASE-FORMING QUALITY OF FERTILIZERS

By L. E. Horat (Department of Agricultural Chemistry, Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*

The referee work on this subject for the past year was concerned chiefly with the tentative method for the determination of acid- and base-forming quality of fertilizers, *Methods of Analysis, A.O.A.C.*, 1935, 34–35. In accordance with the recommendations of the Association, *This Journal*, 21, 62 (1938), and in view of the development of a new mixed indicator by Pierre, Tully, and Ashburn,[1] a collaborative study of the tentative method was made with special reference to a comparison of the old and newly proposed indicators.

The use of 0.5 N sodium hydroxide solution in place of 1.0 N sodium hydroxide solution in the titration and the use of a filter paper cone to prevent loss by spattering, *This Journal*, 21, 301 (1938), during the ignition were also included in this collaborative study.

The four fertilizer samples prepared for the collaborative work are listed in Table 1, together with pertinent analytical data. Sample C-1 is a high analysis superphosphate; Sample C-2 is a representative complete fertilizer made non-acid forming by the addition of limestone; and Sample C-3 is another complete fertilizer with its acidity uncorrected. Sample C-4 is the ordinary Tennessee Brown Rock phosphate. All the material was ground to pass a 40-mesh sieve to insure more uniform and representative analytical samples. In view of previous experience by the Associate Referee these samples furnish a thorough test of the tentative method and proposed modifications.

TABLE 1.—*Samples for collaborative analysis*

SAMPLE NUMBER	FERTILIZER ANALYSIS	INSOLUBLE P₂O₅	TOTAL N	WT. OF SAMPLE TO BE USED	TOTAL ACIDITY CORRECTION
		per cent	*per cent*	*gram*	*lbs. CaCO₃/ton*
C-1	0-44-0	1.3		0.5	36.7
C-2	2-12-6	1.6	2.4	1.0	130.8
C-3	4-12-4	1.1	4.4	1.0	188.1
C-4	Tenn. Br. Rock	31.7		0.5	893.9

To save time and for the sake of uniformity, it was directed that collaborators use the above total acidity corrections in the calculation of their results and the indicated weights of samples.

DIRECTIONS TO COLLABORATORS

The following directions were submitted to the collaborators:

(1) Determine the acid- or non-acid-forming quality as directed under **55,**

[1] *Ind. Eng. Chem. Anal. Ed.*, 10, 72 (1938).

pages 34 and 35, *Methods of Analysis, A.O.A.C.,* 1935. Use 0.5 *N* NaOH in place of 1.0 *N* NaOH in every case.

(2) Proceed as directed in (1) except as indicator use 10 drops of 1% bromophenol blue in 50% ethyl alcohol. Titrate to the color change (corresponding to *p*H 4.3–4.5) from yellow or orange magenta to grey or blue magenta when observed by transmitted light through a thin layer of solution as around the edges of a tipped Erlenmeyer flask. This color change occurs just before the final change to definite blue.

(3) Proceed as directed in (1) except as an indicator use the following: Weigh 0.1 gram of bromocresol green and 0.02 gram of methyl orange into an agate mortar, grind with small amounts of NaOH, using a total of 2 cc. of 0.25 *N* NaOH or its equivalent and make up to 100 cc. volume with water. Use 10 drops of solution per 150 cc. of solution titrated. Titrate to a light green color (*p*H 4.3), described as where the green definitely predominates over the yellow. For further details see *J. Ind. Eng. Chem. Anal. Ed.,* 10, 75 (1938).

In one of the duplicate determinations under (1), (2), or (3) for each sample use a filter paper cone to minimize spattering as follows: After addition of the Na_2CO_3-sucrose solution to the sample in the beaker and before evaporation on the sand bath, insert a filter paper (low ash) cone folded so the base will just slip into the beaker, rest on the bottom, and touch the sides all the way around. Cut off the apex of the cone to form an open vent about 3 mm. in diameter.

Please mark (with asterisk or similar sign) on report blank those determinations where filter paper cone was used. If used in all determinations please note on report blank.

If convenient, a determination of the *p*H of the average end point obtained with each indicator will be of additional value.

In case all samples can not be completed it is recommended that one, two, or three samples be run through with each indicator.

Your observations, opinions, and preferences will be especially valuable.

RESULTS OF COLLABORATORS

In Table 2 the equivalent acidities or basicities for each sample, as determined by each collaborator by both methods, are given in pounds of calcium carbonate per ton of fertilizer. The results represent the average values of two or more separate determinations. A summary of the results in Table 3 shows a comparatively small difference in values obtained with each of the three indicators.

COLLABORATORS*

1. W. A. Morgan, Wilmington, Del.
2. R. L. Jones, Navassa, N. C.
3. H. C. Batton, Baltimore, Md.
4. Paul Caldwell, East St. Louis, Ill.
5. E. W. Cowan and L. D. Haigh, Columbia, Mo.
6. Henry A. Davis, Durham, N. H.
7. Oscar I. Struve, Buffalo, N. Y.
8. R. D. Caldwell, Atlanta, Ga.
9. L. V. Rohner, Syracuse, N. Y.
10. Frank O. Lundstrom, Washington, D. C.

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* Of the 37 collaborators submitting reports, 20 are from the laboratories of fertilizer or allied companies, 16 are from Experiment Stations or similar institutions, and one is from a commercial laboratory.

TABLE 2.—*Collaborative results on determination of acidity and basicity of fertilizers*

	SAMPLE C-1				SAMPLE C-2		
NUMBER OF COLLABORATOR	METHOD			NUMBER OF COLLABORATOR	METHOD		
	1	2	3		1	2	3
1	100B	100B	94B	1	21A	16A	17A
2	6B	5B	10B	2	62A	61A	66A
3	31A	1A	28A	3	92A	100A	105A
4	22A	28B	7A	4	73A	61A	101A
5	16A	22B	24A	5	103A	70A	77A
6	40A	7A	2A	6	91A	64A	68A
7	51A	22A	24A	7	107A	80A	80A
8	37A	34A	27A	8	83A	58A	78A
9	71A	29B	11A	9	139A	106A	94A
10	53A	27A	20A	10	98A	77A	64A
11	25B	25B	40B	11	54A	47A	59A
12	23A	1B	28A	12	73A	63A	71A
13	9A	9A	2A	13	77A	71A	82A
14	9A	3A	3A	14	76A	86A	76A
15	22A	2A	9A	15	81A	63A	51A
16	17A	27A	27A	16	86A	71A	81A
17	67A	87A	47A	17	116A	104A	94A
18	6B	17B	26B	18	75A	78A	75A
19	18B	38B	18B	19	58A	58A	53A
20	13A	1B	13B	20	75A	58A	70A
21	4A	3A	1A	21	78A	67A	75A
22	33B	86B	83B	22	48A	22A	21A
23	137A	112A	87A	23	146A	91A	141A
24	65B	12B	24B	24	86A	74A	68A
25	3B	63B	23B	25	71A	1A	36A
26	13A	8A	7A	26	89A	70A	78A
27	32A	2B	14B	27	98A	36A	62A
28	2A	58B	2A	28	51A	41A	31A
29	29B	34B	29B	29	55A	30A	78A
30	32B	48B	30B	30	54A	41A	61A
31	4A	13A	9A	31	77A	76A	68A
32	37A	22A	37A	32	76A	48A	81A
33	42A	17A	22A	33	96A	140A	128A
34	56A	26B	33B	34	83A	10A	32A
35	36A	35A	38A	35	68A	60A	53A
36	14A	27A	17A	36	68A	70A	74A
37	91B	103B	113B	37	17A	7A	1A
Av. Acidity or Basicity (lbs. CaCO₃ per ton)	12A	7B	2B	Av. Acidity (lbs. CaCO₃ per ton)	80A	63A	71A
Av. Deviation	30	32	28	Av. Deviation	18	20	18
Max. Deviation	125	119	111	Max. Deviation	66	77	70

TABLE 2.—*Collaborative results on determination of acidity and basicity of fertilizers—Continued*

NUMBER OF COLLABORATOR	SAMPLE C-3			NUMBER OF COLLABORATOR	SAMPLE C-4		
	METHOD				METHOD		
	1	2	3		1	2	3
1	319A	303A	303A	1	254B	370B	347B
2	356A	353A	365A	2	246B	248B	217B
3	342A	347A	337A	3	151A	116A	156A
4	266A	341A	356A	4	114A	71B	11B
5	383A	351A	362A	5	67A	103B	87B
6	368A	348A	357A	6	38B	27B	56B
7	392A	375A	373A	7	53B	102B	94B
8	396A	331A	371A	8	36B	81B	26B
9	412A	381A	385A	9	126A	78B	74B
10	380A	366A	362A	10	73A	99B	29B
11	348A	341A	347A	11	53B	101B	97B
12	359A	347A	349A	12	59B	104B	88B
13	362A	366A	369A	13	54B	59B	59B
14	358A	366A	357A	14	266B	273B	261B
15	361A	351A	343A	15	71B	99B	104B
16	378A	378A	368A	16	127B	120B	115B
17	391A	388A	381A	17	6B	21B	1B
18	363A	348A	350A	18	64A	16B	3B
19	335A	338A	346A	19	61B	71B	51B
20	359A	351A	352A	20	80B	112B	78B
21	366A	354A	367A	21	56B	53B	61B
22	341A	307A	298A	22	126B	4B	186B
23	421A	368A	413A	23	14A	66B	6B
24	388A	357A	356A	24	124B	202B	215B
25	371A	318A	341A	25	31B	156B	61B
26	368A	345A	373A	26	43B	50B	36B
27	383A	342A	353A	27	66A	130B	59A
28	321A	316A	338A	28	217B	296B	226B
29	340A	310A	353A	29	142B	272B	117B
30	338A	328A	354A	30	142B	170B	163B
31	361A	362A	369A	31	—	—	—
32	371A	351A	358A	32	—	—	—
33	363A	366A	363A	33	111B	16B	131B
34	379A	332A	327A	34	62A	—	—
35	363A	373A	360A	35	110B	117B	118B
36	360A	357A	357A	36	—	—	184B
37	298A	295A	293A	37	194B	186B	179B
Av. Acidity (lbs. CaCO₃ per ton)	360A	350A	354A	Av. Basicity (lbs. CaCO₃ per ton)	58B	108B	90B
Av. Deviation	20	16	14	Av. Deviation	81	60	62
Max. Deviation	94	55	61	Max. Deviation	209	262	257

TABLE 3.—*Comparison of indicators as shown by average value of collaborators' results*

| SAMPLE NUMBER | EQUIVALENT ACIDITY OR BASICITY VALUES (POUNDS CaCO₃/TON) | | | |
	METHYL RED (1)	BROMOPHENOL BLUE (2)	BROMOCRESOL GREEN* M.O.	ELECTROMETRIC* pH 4.5
C-1	12A	7B	2B	18A
C-2	80A	63A	71A	60A
C-3	360A	350A	354A	367A
C-4	58B	108B	90B	184B

* Obtained by interpolation of values used in plotting curves of Fig. 1.
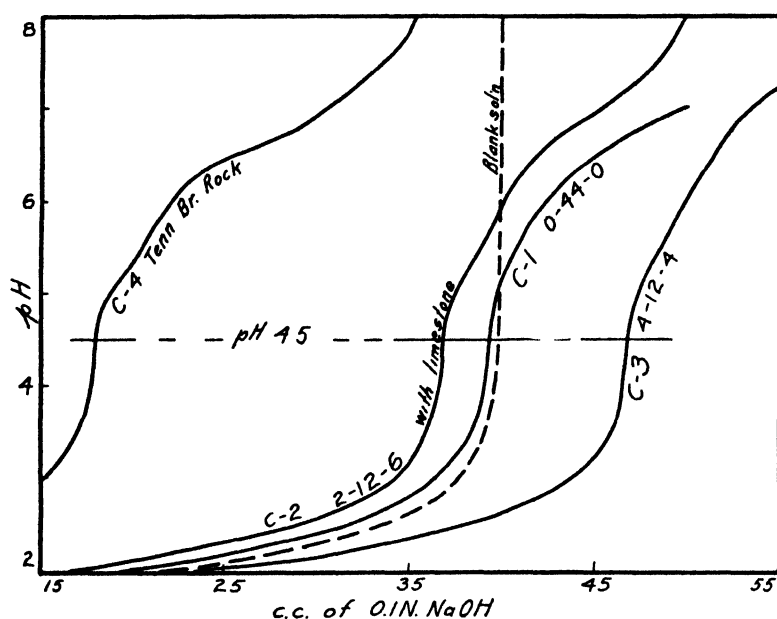
FIG. 1.—TITRATION CURVES OF FERTILIZER SAMPLES
(READINGS MADE WITH BECKMAN pH METER)

11. Mary C. Fox and C. Clifton Howes, Baltimore, Md.
12. R. C. Koch, Hammond, Ind.
13. G. S. McDaniel, Atlanta, Ga.
14. J. Preston Yarborough and Chas. Buchwald, Atlanta, Ga.
15. J. G. McCallister, Jr., Baltimore, Md.
16. Carl Neutzel, Baltimore, Md.
17. G. S. Fraps and T. L. Ogier, College Station, Tex.
18. H. R. Allen, Lexington, Ky.
19. C. L. Hare and T. H. Burton, Auburn, Ala.
20. P. J. Buchanan and R. C. Charlton, Baltimore, Md.
21. C. A. Butt and C. M. Cartledge, East Point, Ga.
22. Robt. P. Thornton, Tampa, Fla.

23. W. H. MacIntire and L. J. Hardin, Knoxville, Tenn.
24. Wm. C. Geagley and Mack M. Nasif, Lansing, Mich.
25. L. S. Walker and E. F. Boyce, Burlington, Vt.
26. Joe J. Scherer, Tallahassee, Fla.
27. Gordon Hart, Tallahassee, Fla.
28. C. R. Byers, Carteret, N. J.
29. Geo. E. Grattan and C. V. Marshal, Ottawa, Canada.
30. John B. Smith and D. R. Willard, Kingston, R. I.
31. W. R. Austin, Nashville, Tenn.
32. F. B. Carpenter and H. L. Moxon, Richmond, Va.
33. H. D. Haskins and A. F. Spelman, Amherst, Mass.
34. Geo. F. Moore and Thos. Beer, Tampa, Fla.
35. E. W. Magruder and W. A. Ryder, Norfolk, Va.
36. W. H. Pierre and R. W. Pearson, Ames, Iowa.
37. J. Morrisson, Chicago Heights, Ill.

The average deviation values summarized in Table 4 show that in case of three of the four samples the mixed indicator of bromocresol green and methyl orange gave more concordant results than did either methyl red or bromophenol blue.

TABLE 4.—*Comparison of indicators as shown by deviations from average values**

SAMPLE NUMBER	DEVIATION FROM AVERAGE VALUE FOR EACH INDICATOR (POUNDS CaCO$_3$/TON)					
	METHYL RED (1)		BROMOPHENOL BLUE (2)		BROMOCRESOL GREEN—M.O. (3)	
	AV.	MAX.	AV.	MAX.	AV.	MAX.
C-1	30	125	32	119	28	111
C-2	18	66	20	77	18	70
C-3	20	94	16	55	14	61
C-4	81	209	60	262	62	257
Av. Dev.	37		32		31	
Max. Dev.		124		128		125

* Average of all collaborative values for each sample.

COMMENTS OF COLLABORATORS

In response to the questionnaire sent to each collaborator, many valuable observations, opinions, and preferences in regard to various technical details of the method were obtained. In addition, several collaborators submitted experimental evidence of probable sources of error in the tentative method.

The recommendation of the Association that 0.5 N sodium hydroxide solution be used in the titration in place of 1.0 N sodium hydroxide solution, was endorsed by 30 out of 37 collaborators. Six preferred weaker alkali and only one favored stronger alkali. The preference for indicators was decidedly in favor of Pierre's mixture of bromocresol green and methyl orange.

The use of a filter paper cone for the prevention of spattering during drying and ignition of the sample was definitely favored by a significant

majority of the collaborators. Very few considered its use unnecessary and they usually avoided spattering by slow and carefully controlled or constantly attended evaporation. Many emphasized the greater accuracy and the increased speed of evaporation or drying possible when the cone is used. As a means for initial evaporation of the sample and sodium carbonate-sucrose solution, the sand bath and electric hot plate were about equal in preference among the collaborators. The temperatures favored were an initial one of 100°–120° C. up to 150°–200° C. final temperature before ignition in a furnace.

The final volume of the filtrate when titrated varied among the collaborators from 100 cc. to 250 cc., with 150 cc. the approximate volume titrated by a majority of the collaborators. This item is significant in some samples high in phosphate and lime in that the larger volume or more dilute solution decreases the possibility of interference by precipitation or cloudiness near the end point.

A matter of special importance in regard to its effect on results obtained by the present tentative method is the acid extraction of the ashed sample prior to filtration and titration. Although directions in the tentative method are explicit on this point, evidence was submitted in 1936 by C. L. Hare of Alabama showing the acid extraction of the ashed residue to be incomplete in the case of some samples. By means of a second extraction with additional acid, Hare found nine different fertilizers which yielded from 10 to 25 pounds calcium carbonate additional basicity. He suggested that this additional basicity might be due to larger particles of limestone (10–20-mesh fineness). However, with Sample A-5 used in the 1936 collaborative study, he obtained from 65 to 100 pounds of calcium carbonate additional basicity by a second acid extraction and this sample was ground to pass a 40-mesh sieve before being sent out by the Associate Referee. Hence it appears that during the ashing or ignition of the sample coarser basic aggregates are formed and that they are not completely dissolved during the acid extraction as prescribed in the tentative method. This year Collaborators Butt and Cartledge, using a 0.5 gram sample (C-4, Tennessee brown rock phosphate) as directed by the Associate Referee, submitted data showing approximately 200 pounds of calcium carbonate additional basicity obtained by extracting the ignited sample with 40 cc. of 1.0 N hydrochloric acid instead of 30 cc. of 1.0 N hydrochloric acid, as prescribed in the tentative method. According to the analysis of Sample C-4 and the present tentative method, its available phosphoric acid content of 3–4 per cent would call for the use of a 1 gram sample. However, in view of his past experience as well as that of Hare, the Associate Referee directed the use of a 0.5 gram sample in the hope of overcoming the above difficulty. In a recent communication this difficulty with Sample C-4 was confirmed by Pierre and Pearson. Pierre suggests sufficient excess acid

and sufficient time for digestion of samples that are very basic or where a large amount of coarse dolomitic limestone or ground bone is present.

ELIMINATION OF WATER-INSOLUBLE MATERIAL COARSER THAN 20-MESH

During the past year over 300 unground samples of commercial mixed fertilizers of all the more common analyses have been collected for an investigation by wet sieving to determine whether any fertilizers or class of fertilizers contain sufficient water-insoluble material coarser than 20-mesh of sufficient basicity to justify elimination of this portion before the tentative method is applied. Taylor and Pierre[2] found that these coarser portions of dolomitic limestone were not available during the year of application. This work by the Associate Referee has not been completed.

CALCULATION OF RESULTS

The directions for calculating results according to the present tentative method seem to be explicit, but the difficulty experienced by some collaborators both this year and in 1936 induced the Associate Referee to submit a formula to all collaborators who had been asked to recheck their calculations when submitted results were very much out of line with the average. In response to solicited opinions regarding the use of such a formula, many collaborators submitted formulas that had been used in their laboratories. Below is a composite of the various formulas proposed:

$$\left\{ \frac{(\text{cc. } 0.5 \ N \text{ NaOH blank} - \text{cc. } 0.5 \ N \text{ NaOH sample}) \times 50 \text{ lbs. CaCO}_3}{\text{Wt. of sample used in grams}} \right\}$$

$$\text{minus } (\% \text{ Total N} \times 35.7) \text{ minus } (\% \text{ insol. P}_2\text{O}_5 \times 28.2)$$

When the formula is solved algebraically, a positive result indicates that the sample is basic or non-acid forming, while a negative result indicates sample is acid or acid-forming.

All collaborators who misinterpreted the directions for calculating results in the tentative method favored substitution of a formula of the above type.

RECOMMENDATIONS[3]

It is recommended—

(1) That in the present tentative method for the determination of acid- and base-forming quality of fertilizer, *Methods of Analysis, A.O.A.C.,* 1935, methyl red indicator, 34, 55(a), be replaced by the mixed indicator prepared by weighing 0.1 gram of bromocresol green and 0.02 gram of methyl orange into an agate mortar, and triturating while slowly adding

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[2] *J. Am. Soc. Agron.,* **27,** 764 (1935).
[3] For report of Subcommittee A and action by the Association, see *This Journal,* **22,** 53 (1939).

about 2 cc. of 0.1 N NaOH, and then diluting to 100 cc. with water (first action).

(2) That in the same method 0.4 cc. of the mixed indicator be used in titration in place of methyl red (first action).

(3) That the same method be modified by the substitution in the titration of 0.5 N sodium hydroxide in place of 1.0 N sodium hydroxide solution (final action).

(4) That the same method be modified by making optional the use of a filter paper cone for the prevention of spattering (first action). This cone should be of low ash paper and such size that when folded the base will just slip into the beaker, rest on the bottom, and touch the sides all around. The apex is cut off to provide a vent of about 3 mm. diameter, *This Journal*, 21, 301 (1938).

(5) That in the same method the elimination of water-insoluble material coarser than 20 mesh before the method is applied be studied further.

(6) That the basicity of phosphate rock and other factors that affect the method be studied further.

(7) That in the same method, p. 34, in 55(b), the typographical error of "286 g of $Na_2CO_3 \cdot H_2O$" be corrected to read "286 g $Na_2CO_3 \cdot 10H_2O$."

The paper, entitled "A Comparison of the Official and MacIntire-Shaw-Hardin Methods for Determining Available P_2O_5," by J. Richard Adams, is published in this number of *This Journal*, p. 397.

The paper, entitled "Citrate Solubility of the Magnesium in Dolomite of Varying Particles Size," presented by Whittaker, Rader, and Zahn, was published in *This Journal*, 22, 180 (1939).

REPORT ON CALCIUM, SULFUR, COPPER, AND ZINC

By Gordon Hart (Chemical Division, Agricultural Department, Tallahassee, Florida), *Associate Referee*

The Bartlett-Tobey method for the determination of calcium was tried out in this laboratory by W. Y. Gary. The results appear to be very promising, and bromophenol blue seems a better indicator than methyl red. If the precipitation is made at pH 3.5–4.0, iron and alumina do not interfere, but manganese may interfere if there is much present.

It is recommended[1] that collaborative work on methods for the determination of calcium, sulfur, copper, and zinc be done.

The paper, entitled "Improved Molybdenum Blue Reagents for Determination of Phosphorus and Arsenic," by J. A. Schricker and P. R. Dawson, was published in *This Journal*, 22, 167 (1939).

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 51 (1939).

REPORT ON EGGS

By H. A. LEPPER (U. S. Food and Drug Administration,
Washington, D. C.), *Referee*

The investigations on eggs this year were curtailed by the press of other duties on the part of some of the associate referees.

It is recommended[1]—

(1) That the title of par. 16, page 301, *Methods of Analysis, A.O.A.C.,* 1935, be changed to read, "Chlorine," and that the directions be revised as recommended by the associate referee (see *This Journal*, **22,** 77 (1939).

(2) That the method for the determination of dextrose and sucrose, page 301, **18,** be amended as suggested by the Associate Referee, *ibid.*

(3) That the studies on methods for dried eggs be discontinued.

(4) That study of methods for glycerol be continued.

(5) That study of methods for decomposition be continued.

(6) That study of the method for the determination of cholesterol and fat be continued.

No report on unsaponifiable constituents and fat was given by the associate referee.

REPORT ON DETECTION OF DECOMPOSITION

By J. CALLAWAY, JR. (U. S. Food and Drug Administration,
Washington, D. C.), *Associate Referee*

The Associate Referee believes that there is a real need for more official chemical methods for the measurement of decomposition in eggs. In order to be of greatest use such methods should not require elaborate apparatus or excessive time. A rapid method for the determination of acidity of ether extract in liquid eggs has been adopted as tentative, and last year a rapid method for determination of ammonia nitrogen was submitted to collaborators. The latter method has the advantage of less complicated apparatus and also requires less attention than does the present tentative method. The final results, however, were not identical with those obtained by the tentative method, although there appeared to be a definite relationship between them.

Believing that more work was justified, the Associate Referee requested collaborators to study previous reports and to try and improve the absorption apparatus. After they had prepared and tested such apparatus they were requested to determine ammonia nitrogen by a new rapid method and by the present tentative aeration method on several samples

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, **22,** 61 (1939).

of eggs, including both good and bad. At the suggestion of Shupe it was further directed that sodium or potassium fluoride be added to egg samples in the absorption apparatus to prevent possible increased decomposition during absorption of ammonia.

Reports were received from Manuel Tubis, Food and Drug Administration, Philadelphia, Pa., and Irwin S. Shupe, Food and Drug Administration, Kansas City, Mo., the same collaborators who reported last year. Tubis reports that he tried out a different type of absorption cell consisting of a Petri dish with a partition dividing the dish into two equal compartments. The dish was covered with a piece of ground-glass greased with vaseline. The dimensions of the dish were as follows: diameter 9.5 cm., height 2 cm., height of partition 1 cm. Each compartment had a capacity of about 29 cc. Recovery of ammonia from a standard solution of ammonium salt with this cell was practically 100 per cent.

The acid used was 0.005 N and it contained the mixed indicators recommended by Bandemer and Shaible.[1] The alkali was 0.0025 N. All the saturated potassium carbonate solution was also saturated with sodium fluoride, Tubis further reports.

The results presented (Table 1) were obtained on samples bought in

TABLE 1.—*Results obtained with A.O.A.C. and absorption methods*

	MG. AMMONIA NITROGEN PER 100 GRAMS EGG	
SAMPLE	A.O.A.C. METHOD	ABSORPTION METHOD
1[a]	1.98	3.08
1[b]	2.72	3.18†
2[a]	2.67	2.94
2[e]	2.88	4.01
3	3.04	4.15
3[e]		3.21
3[w]		3.35
4 (Yolk)	6.06	7.39
4 (White)	2.56	1.43[p]

[a] Kept refrigerated.
[b] Kept at room temperature.
† This difference only amounts to 0.02 mg./5 grams higher than by aeration.
[e] These determinations were made a day later on the refrigerated samples.
[w] Sample same as 3[e] but had 3 cc. of water added to the egg, showing dilution caused a slight change.
[p] This is lower than the corresponding result by aeration.

the open market, broken out in the laboratory, and mixed by shaking with glass beads. All were normal in appearance, and none had an abnormal odor.

Sample 1 was divided into two equal parts; one part, 1[a], was kept refrigerated, and the other, 1[b] was left in the laboratory at room temperature to induce a slight spoilage.

[1] *Ind. Eng. Chem. Anal. Ed.*, **8**, 201 (1936).

In another sample, the use of 4 cc. of saturated carbonate instead of 3 cc. gave a result of 0.03 mg./100 grams higher.

The new dish was compared with the old cell (Table 2). The samples used had been diluted to about twice their original volume.

TABLE 2.—*Comparison of "new dish" and "old cell" methods*

SAMPLE	NEW DISH	OLD CELL
	mg./100 grams	
5	3.38	3.45
"Fresh egg"	2.86	2.77
"Second grade egg"	3.24	3.07
6	2.48	2.41
7	3.30	3.28
7*	3.76	3.96
Control (recoveries)	100%	100%

* Incubated for 6 hours at 37.5° instead of usual 5 hours.

The "new dish" method was compared with the aeration method on three subdivisions of a sample of commercial frozen eggs. The results are shown in Table 3.

TABLE 3.—*Comparison of "new dish" and aeration methods*

SUBDIVISION	MG. AMMONIA NITROGEN/100 GRAMS	
	NEW DISH	AERATION
1	2.62	2.12
2	2.84	2.25
5	2.32	2.14

Summarizing, the aeration method gives slightly lower results than the proposed method, and the "new dish" method tried gives comparable but slightly higher results than the proposed method.

Shupe also tried some modifications of the absorption apparatus. He found that a glass cylindrical cell divided in half was not so efficient as the one he had used the year previously, which was of the type recommended by Bandemer and Schaible.[1] He recommends that the inner cell have a clearance of at least 4 mm. from the glass plate used as cover.

Shupe reports as follows on work using the adsorption apparatus just described:

Two samples of whole eggs (A and B) were analyzed by the A.O.A.C. tentative aspiration method and by the absorption method. For the proposed rapid method 5 grams (+0.05) samples were weighed into the absorption cells and 3 cc. of water was mixed with the sample. A measured volume of standard 0.02 N acid, 5 cc. for B and 2 cc. for A, was added to the inner cell. The cover-glasses were put in place and 3 cc. of saturated potassium carbonate solution was added to the egg in the outer chamber. The cells were then allowed to stand 5 hours at room temperature

(27°–30° C.). Methyl red indicator and 0.02 N acid and alkali were used. The results obtained are given in Table 4.

TABLE 4.—*Results by proposed rapid absorption method*

SAMPLE	NH₃/100 GRAMS
A—without NaF	3.0
A—K₂CO₃ solution saturated with NaF	3.0
A—K₂CO₃ solution saturated with NaF	3.4
B—without NaF	23.2
B—without NaF	23.6
B—K₂CO₃ solution saturated with NaF	21.1
B—K₂CO₃ solution saturated with NaF	20.9

* STANDARD (NH₄)₂SO₄ EQUIVALENT TO MG. NH₃ AS FOLLOWS:	RECOVERY IN 5 HOURS AT ROOM TEMPERATURE
	per cent
0.34 (with NaF)	100
0.68 (with NaF)	95
1.02 (with NaF)	93
1.70 (with NaF)	96

SAMPLE	NH₃/100 GRAMS
	mg.
A—By A.O.A.C. Aspiration	2.9
B—By A.O.A.C. Aspiration	19.1

* Standards contained 1 cc. of 0.02 N acid in excess of amount required to neutralize the ammonia.

REMARKS: Slightly higher results were obtained by the absorption method than by the A.O.A.C. aspiration method. 5 hours at room temperature (27°–30° C.) seems to be an adequate time and high enough temperature for the absorption of the ammonia.

The use of sodium or potassium fluoride seems advisable.

CONCLUSION

The results of the collaborators show that the absorption method gives slightly higher results than does aeration. Since the aeration method requires a special set-up and close attention it becomes very time consuming when many samples are to be examined. The absorption method requires less apparatus and less attention.

The Associate Referee believes that more work on standardization of absorption apparatus should be done. He believes that on the whole the absorption method is preferable to the aeration method.

RECOMMENDATIONS[1]

It is recommended—

(1) That further work be done on the absorption method for the determination of ammonia nitrogen in liquid eggs.

(2) That additional chemical methods for measurement of decomposition in eggs be sought.

REPORT ON ADDED GLYCEROL, SUGAR, AND SALT

By L. C. MITCHELL (U. S. Food and Drug Administration,
Minneapolis, Minn.), *Associate Referee*

GLYCEROL

No collaborative work was done on the methods for the detection and determination of added glycerol in yolks. As the tentative method for the determination of glycerol is not applicable in the presence of sugars, the Associate Referee did some preliminary work on the possibility of separating glycerol from sugar by distillation with superheated steam (up to 235° C.), but found that the glycerol did not completely distil. Time did not permit further work.

SALT

The collaborators were asked to add 10 per cent previously dried salt to egg white and proceed as directed under XXIII, 16, *Methods of Analysis, A.O.A.C.*, 1935, correcting the results by subtracting the amount of salt found in the blank, which salt is due to the naturally occurring chlorine compounds found in eggs. The results are given in Table 1.

TABLE 1.—*Results on 10% added salt in egg white*

COLLABORATOR	SALT RECOVERED
	per cent
Donald A. Ballard, Food and Drug Administration, Seattle	10.02
	10.02
Edward O. Haenni, Food and Drug Administration, Washington	9.98
	9.96
O. S. Keener, Food and Drug Administration, Cincinnati	10.14
	10.00
	10.01
G. E. Keppel, Food and Drug Administration, New Orleans	9.95
	9.97
C. A. Kuehl, H. J. Heinz Company, Pittsburgh	9.93*
	9.99*
J. A. Mathews, Food and Drug Administration, Washington	10.02
	10.00
Average	10.00

* Gravimetric method. All other results volumetric.

SUGAR

The official method for the determination of dextrose and sucrose in eggs, *Methods of Analysis, A.O.A.C.*, 1935, XXIII, 18, has been found satisfactory, *This Journal*, 14, 397 (1931); 16, 74, 305 (1933), with the exception of the error due to the volume of the precipitate, which is appreciable in liquid yolks, dried yolks, or whole eggs. The errors may

be corrected by such well-known methods as (1) double dilution, (2) filtration, followed by washing the residue free from sugar and making filtrate up to a given volume, and (3) correction of the volume occupied by the precipitate.

Method (3) means that corrections must be made for the volume occupied by the added calcium carbonate (probably a constant), by the proteins, and by the fat, with the percentages of the proteins and fat varying with each sample. Fat is readily determined, so is the nitrogen, but further correction would be necessary on the nitrogen since some of it is found in the fatty substances (lecithin). Thus far the Associate Referee has made no headway in establishing a correction factor for the volume occupied by the precipitate. Method (2) was abandoned because prolonged washing caused some of the residue to pass through the filter. Method (1) was found to yield promising results.

The collaborators were accordingly asked to add 10 per cent previously dried sucrose to yolks and proceed as directed under XXIII, 18, *Methods of Analysis, A.O.A.C.,* 1935; and to run a second determination, using the same amount of sample and calcium carbonate, but twice the amount of salt solution and alcohol and making the volume to 500 cc. instead of 250 cc. as given in the method. To correct the error due to the volume occupied by the precipitate, they were also requested to subtract the percentage of sucrose found in the 250 cc. determination from twice that found in the 500 cc. The results are given in Table 2.

TABLE 2.—*Results on 10% added sucrose in egg yolks*

COLLABORATOR	SUCROSE RECOVERED
	per cent
Edward O. Haenni	10.05
	10.07
O. S. Keener	9.93
	9.94
G. E. Keppel	9.88
	9.99
C. A. Kuehl	9.83
J. A. Mathews	9.90
	9.98
Average	9.95

COMMENTS OF COLLABORATORS

Ballard.—Added salt. The method as outlined under XXIII, 16 (a), *Methods of Analysis, A.O.A.C.,* 1935, was used for the natural salt in the whites. However, for the sample the filtrate from the charred mass was diluted to 200 cc. and a 40 cc. aliquot used for the determination, since it was obvious that the entire filtrate would require an excessively large quantity of silver nitrate. The duplicate analyses reported are from separate 10-gram portions of whites, each treated according to the method with the above noted exception.

Haenni and Mathews.—In the method for the determination of sucrose we encountered difficulty in making the mixture to the mark in the flasks, due to the entrapment of considerable volumes of air in the precipitate. We found it advisable to remove this air by applying suction to the mixture before making it to volume, the addition of a few drops of alcohol and ether being used to facilitate this operation. The difficulty seems to be particularly great in the case of the 250 cc. dilution.

The method for the determination of chlorine in eggs and egg products, as given in *Methods of Analysis,* is even yet somewhat ambiguous since the reference to the final determination calls for filtering off and washing the excess silver chloride, whereas the solution is obviously prepared with the intention of back titrating an aliquot of the excess silver nitrate. In the present work the chlorine in the egg white itself was determined according to the procedure in XXIII, 16, 17, an aliquot of the solution containing the excess silver nitrate being back titrated. The egg white containing added salt was analyzed similarly, an aliquot of the prepared solution being used for the precipitation of the chlorine.

Would it not be possible to establish a correction factor based on this double dilution method, correlated, perhaps, with the total solids or other determination, so that the double determination involved in this procedure would not be necessary in routine work?

DISCUSSION

Collaborative work was reported in 1932, *This Journal,* 16, 298 (1933), for added salt and sugar in whole eggs. At that time no correction was made for any error due to the volume occupied by the precipitate in the sugar determination, as such error is not particularly large. This error, however, is appreciable in such products as yolks, dried yolks, dried whole eggs, or frozen yolks, the latter product being the one in which added sugar is most likely to be encountered.

The results for both added salt and sugar are well within the analytical error of the respective methods, and are remarkably close to the theoretical.

Collaborator Haenni called attention to the ambiguity of the method for preparation of solution and to the fact that the reference to XII, 35 (gravimetric) in the determination should be XII, 37 (volumetric). In order to clarify the procedure and to include samples containing added salt, the method has been modified for consideration of the Association. (The modified method was published in *This Journal,* 22, 77 (1939).)

In the presence of added salt, correction is made for the natural chlorine-bearing substances present in the type of egg product under examination. For eggs (hen) the corrections are approximately as follows, *This Journal,* 15, 310 (1932): 0.3 per cent on liquid eggs (whether yolks, whites, or whole eggs); 0.6 per cent on dried yolks, 1.1 per cent on dried whole eggs, or 2.4 per cent on dried whites (albumin) for the naturally occurring chlorine-bearing substances calculated as salt.

It is suggested for the consideration of the Association that the method proposed for correction of error due to the volume of the precipitate in samples containing added sugar be incorporated at the end of the para-

graph **18(a)**, These directions were published in *This Journal*, **22, 77** (1939).

The directions for inversion of sucrose in the method, XXIII, **19,** are those used by the Association for many years, but they do not now conform to the general method for sugars. Accordingly, Recommendation (3) is offered for the consideration of the Association.

RECOMMENDATIONS[1]

It is recommended—

(1) That the official method for the determination of chlorine, *Methods of Analysis, A.O.A.C.,* 1935, XXIII, **16,** be modified along the lines suggested by the Associate Referee.

(2) That the official method for the determination of dextrose and sucrose, **18,** be modified as suggested by the Associate Referee and adopted as official (first action).

(3) That the words "add 5 cc. of HCl, and allow to stand overnight" under XXIII, **19,** Reducing Sugars Invert, be deleted and that the following words, "and invert the sucrose as directed under XXXIV, **23(b)** or **(c),**" be inserted.

(4) That the study of methods for glycerol be continued.

No report on dried eggs was given by the associate referee.

REPORT ON PRESERVATIVES

By WILLIAM F. REINDOLLAR (State of Maryland Department of Health, Baltimore, Md.), *Referee*

The special method for the determination of saccharin in non-alcoholic beverages was investigated, modified, and submitted to collaborative study, to learn whether or not it is applicable to semi-solid preparations. The results of this investigation indicate that this method is unsuitable for these products. It is recommended,[2] therefore, that further work on this method be discontinued.

REPORT ON BENZOATE OF SODA

By A. E. MIX (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

It was suggested that the Associate Referee study the determination of benzoate of soda in food products, for which a rapid chemical method giving accurate results is required. A number of methods were reviewed

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 61 (1939).
[2] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 66 (1939).

and it is believed that the requirements may be met by a modification of the Monier-Williams method.

The apparatus consists of a boiling flask fitted with reflux (bulb) condenser, a large test tube (constricted at the center) for holding 3 grams of magnesium turnings in the upper portion of the tube, and two pieces of glass tubing connected in such a manner as to fit into a rubber stopper, which closes the neck of the flask and at the same time receives the lower end of the condenser tube.

METHOD

The sample is mixed with water if necessary, and an excess of NaCl is added (40 grams salt per 100 cc. solution). The mixture is acidified with P_2O_5, and 2 cc. is added in excess. A boiling tube is added to the flask, the flask is connected with the condenser, and the sample solution is boiled. This does not require any attention until after 3 hours of boiling. The flask is disconnected, and the Mg benzoate formed in the test tube is extracted with the hot water used for rinsing the inside of the condenser tube and also for the glass tubes attached to the rubber stopper. Thorough extraction is required but the volume of this extraction should be kept as small as possible. (The magnesium turnings placed in a small beaker immersed in a small casserole of boiling water and allowed to heat for 10 minutes were more thoroughly extracted than if left in the tube.) The extracted solution is filtered through glass wool and made strongly alkaline with about 20 drops of a 40% solution of NaOH. It is cooled to 40°–50°C., and oxidized with a saturated solution of KMO_4 until the

Results on benzoate of soda by a modified Monier-Williams method

SAMPLE	NaC₆H₅COO WT. OF CHARGE	WEIGHT RECOVERED	YIELD	DURATION OF BOILING	SOLVENT MIXTURE USED, BEFORE TITRATION
	gram	*gram*	*per cent*	*hours*	
1	0.0594	0.05526	93.0	3	100 cc. H₂O
1A	0.0500	0.04850	97.0	3	25 cc. Et OH, 95%
2	0.0500	0.0513	101.9	3	30 cc. H₂O
					10 cc. Et OH, 95%
3	0.0560	0.0616	110.5	3	100 cc. H₂O
					25 cc. Et OH, 95%
4	0.0500	0.04818	96.4	3	10 cc. H₂O
					30 cc. Et OH, 95%
5	0.0500	0.05784	115.7	3	10 cc. H₂O
					30 cc. Et OH, 95%
6	0.0500	0.05123	102.4	3	10 cc. H₂O
					30 cc. Et OH, 95%
7	0.0500	0.05061	101.2	3	10 cc. H₂O
					30 cc. Et OH, 95%

Nos. 1, 2, 3, sodium benzoate weighed from stock bottle.

Nos. 4, 5, 6, 7, sodium benzoate made into a water solution (1 cc. = .0005 gram); 100 cc. was measured out for sample.

No. 1A, sample of tomato juice to which 0.05 gram of dried Na benzoate was added.

Min. recovery, 93.0% water solution; max. recovery, 115.7% water solution.

Av. recovery, 102.2+.

pink color persists for some minutes. The excess KMO_4 is destroyed by the addition of NA_2SO_3 crystals until an even brown color of MnO_2 is formed. A few drops of concentrated H_2SO_4 are added. The resulting solution is usually clear and colorless. This clear solution is saturated with NaCl and extracted with a 50–50 mixture of ethyl acetate and petroleum ether.

COMMENTS

It appears that 4 or 5 extractions with 25 cc. ethyl acetate and petroleum ether mixture are sufficient.

Violent shaking of separatory funnel should be avoided. The solution was evaporated at room temperature in a current of dry air and allowed to stand overnight (or until no acid odor could be detected).

The residue of benzoic acid was dissolved in various amounts of 95 per cent alcohol and water, 1–2 drops of phenolphthalein indicator were added, and the solution titrated with $0.02N$ NaOH.

Samples 3 and 5 appeared dry but had acid odor and gave high yield. Samples 2, 6, and 7, faintly acid, gave over 100 per cent yield; 1, 1A, and 4, dry, no acid odor, gave reasonable yield.

It appears that drying, retention of acid odor, and the water-alcohol mixture for dissolving the residue must be carefully controlled.

It is recommended[1] that the work on this method be continued and that the method be further studied collaboratively.

REPORT ON COLORING MATTERS IN FOODS

C. F. JABLONSKI (U. S. Food and Drug Administration,
New York City), *Referee*

Last year's recommendations of the Committee requested the Referee to continue the collaborative investigation of the quantitative estimation of Ponceau SX in the presence of Ponceau 3R. With this purpose in view, the Referee sent out to the collaborators six sets of samples, consisting of five subdivisions each, and instructions to estimate the dye mixtures by a submitted method, which was a slight modification of the one tried last year.

The samples in question were of the following composition (based on titanium chloride titrations):

SAMPLE	PONCEAU SX	PONCEAU 3R	TOTAL COLORS
	per cent	*per cent*	*per cent*
1	none	85.38	85.38
2	19.22	67.26	86.48
3	69.46	19.85	89.31
4	90.45	none	90.45
5	48.99	39.19	88.18

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 66 (1939).

The reports of the collaborators as received are as follows:

O. L. Evenson, Food and Drug Administration, Washington, D. C.

SAMPLE	PONCEAU SX	PONCEAU 3R	TOTAL COLORS
	per cent	*per cent*	*per cent*
1	none	83.70	83.70
2	20.70	64.80	85.50
3	66.60	21.30	87.90
4	90.00	none	90.00
5	46.80	39.55	86.35

S. S. Forrest, Food & Drug Administration, Washington, D. C.

1	none	85.30	85.30
2	18.60	64.30	82.90
3	68.40	20.30	88.70
4	90.60	none	90.60
5	48.00	40.80	88.80

Mrs. A. P. Bradshaw, Bureau of Chemistry & Soils, Washington, D. C.

1	none	84.60	84.60
2	21.00	64.60	85.60
3	70.40	18.40	88.80
4	91.80	none	91.80
5	49.70	37.30	87.00

L. Koch, H. Kohnstamm & Co., Brooklyn, N. Y.

1	none	85.40	85.40
2	21.90	65.65	87.55
3	73.25	16.20	89.45
4 (direct)	90.35		90.35
(after treatment)	95.10	none	95.10
5	51.35	36.50	87.85

J. J. Morris, H. Kohnstamm & Co., Brooklyn, N. Y.

1	none	85.67	85.67
2	22.81	65.51	88.32
3	73.24	16.69	89.93
4 (direct)	88.52		88.52
(after treatment)	90.35	none	90.35
5	51.63	36.15	87.78

J. L. Hogan, Food & Drug Administration, New York City.

1	none	84.65	84.65
2	20.71	65.49	86.20
3	67.45	21.30	88.75
4 (before treatment)	88.72		88.72
(after treatment)	86.62	trace or none	86.62
5	47.42	39.31	86.73

The following comments and criticism were offered by the collaborators:

Evenson.—Fairly good check results were obtained in all cases.

Forrest.—The method seems satisfactory, check results being obtained in all cases. Some variation is noted, but in several titrations a majority check closely.

It may be noted that the total titration for Nos. 1 and 2 do not check with those of Evenson. We have checked this point carefully and believe that the difference may be due to moisture absorbed by one of the samples.

Bradshaw.—In Sample 4 the corrected volume after subtracting from the original titration left a value of −0.36 cc. of 0.1 *N* titanium trichloride; this was not considered in the calculations.

Koch and Morris.—As in previous collaboration, the method presented no analytical difficulties. However, the straight ponceau SX Sample 4 consistently resulted in a higher percentage after the peroxide treatment. It is suggested that the collaborative investigation be directed towards the determination of dye mixtures containing 0–20% ponceau SX.

Hogan.—I had no particular difficulties with the method except that the end point while distinct seemed to vary over a range of about 0.20 cc. 0.1 *N* TiCl₃. In Sample 4 the average difference between the unoxidized and oxidized material amounted to 2.10% of dye. This difference may be due to a slight error in the submitted chart.

<div align="center">DISCUSSION</div>

To give a better understanding, the results are summarized as follows (per cent):

Sample

1	Ponceau SX	none	none	none	none	none	none
	Ponceau 3R	83.70	85.30	84.60	84.65	85.67	85.40
2	Ponceau SX	20.70	18.60	21.00	20.71	22.81	21.90
	Ponceau 3R	64.80	64.30	64.60	65.49	65.51	65.65
3	Ponceau SX	66.60	68.40	70.40	67.45	73.24	73.25
	Ponceau 3R	21.30	20.30	18.40	21.30	16.69	16.20
4	Ponceau SX	90.00	90.60	91.80	88.72	88.52	90.35
	Ponceau 3R	none	none	none	none	none	none
5	Ponceau SX	46.80	48.00	49.70	47.42	51.63	51.35
	Ponceau 3R	39.55	40.80	37.30	39.31	36.15	36.50

At first glance the results may not appear to be satisfactory. However, it must be taken into consideration that the color value of Sample 1 and also of Sample 4 as reported by the collaborators showed considerable difference. The extreme reported for Sample 1, which consisted entirely of ponceau 3R, is 1.97 per cent. The extreme reported for Sample 4, which consisted of Ponceau SX, is 3.28 per cent. Whether these differences of the color value of the dyes can be attributed to absorbed moisture cannot be positively answered. It is quite conceivable that mixtures of those dyes would also deviate at least to that extent. Therefore, if due allowances are made for these variations, the submitted results can be considered acceptable, since each 0.1 cc. of 0.1 *N* titanium trichloride represents approximately 0.70 per cent of dye.

In Sample 4 a number of collaborators noted a perceptible difference between color percentage of the treated and untreated sample. The Referee proposes to investigate this phase in the near future.

It is recommended—

(1) That collaborative work be continued on the quantitative estimation of ponceau SX in the presence of ponceau 3R.

(2) That investigational work be continued on the quantitative separation and estimation of tartrazine and sunset yellow FCF in mixtures.

(3) That investigational work be undertaken to separate and estimate quantitatively mixtures of light green SF yellowish, brilliant blue FCF, and fast green FCF.

REPORT ON METALS IN FOODS

By H. J. WICHMANN (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

ARSENIC AND ANTIMONY

Three important papers on the determination of arsenic appeared in 1938 and the early part of 1939. Alfred E. How[2] modified the Gutzeit method in three respects, but still left it an empirical procedure. He substituted a string in a capillary tube for the paper strip, a stick of alloy (zinc 99.5, tin 0.05, lead 0.01, and iron 0.0028 per cent, respectively) for the zinc activated with stannous chloride, and used a double water bath instead of the single bath of the Gutzeit method, providing a difference of 5° C. between the temperature of generator and absorption tube. In addition, he caused the electrolyte to circulate around the stick of alloy. How's results calculate to a standard deviation of 7.4–2.4 per cent for a range of 0.1–100 micrograms, respectively. This is a much better performance than any claimed for the present official Gutzeit method with respect to range, but the accuracy has not been increased proportionally.

Klein and Vorhes[3] recently described the formation and extraction of arsenic ethyl xanthate, insoluble in aqueous acid solutions but soluble in carbon tetrachloride. These authors determine the arsenic finally by the Zinzadze[4] molybdenum blue method with photometric measurement. They applied their method to spray residue quantities of arsenic, leaving to the future the problem of adapting it to smaller quantities. Their standard deviation varied from 3.5 to 1.1 per cent for 100–800 micrograms of As_2O_3. The unusual feature of this paper is the method of arsenic isolation presented. Antimony and tin compounds do not form molybdenum blues, nevertheless these compounds must be removed because of turbidities caused by hydrolysis of their bromides. The removal of the interfering metals is made possible by a difference in the behavior of the

[1] For report of Subcommittee D and action by the Association, see *This Journal*, **22**, 66 (1939).
[2] *Ind. Eng. Chem. Anal. Ed.*, **10**, 226 (1938).
[3] *This Journal*, **22**, 121 (1939).
[4] *Ind. Eng. Chem. Anal. Ed.*, **7**, 230 (1935)!

xanthates in carbon tetrachloride solution, with concentrated hydro-chloric acid. The arsenic xanthate is stable towards that acid and remains in the carbon tetrachloride, but the tin and antimony xanthates, as well as other xanthates that are soluble in carbon tetrachloride or collect at the interface, decompose to the chlorides and dissolve in the acid phase. Here, then, is another possible method for the separation of micro amounts of antimony and arsenic, which should allow their subsequent individual determination.

The third paper, by Cassil and Wichmann,[5] describes a rapid arsine evolution followed by an iodine titration of the arsine. The striking features of this method are the speed of the evolution, the extraordinary efficiency of the arsine absorption in 1 cc. of mercuric chloride solution, and the accuracy of the final micro titration in a small volume of solution. An arsenic determination, exclusive of sample preparation, can be made in 10 minutes, and a standard deviation, constant over a range of 5–500 micrograms of As_2O_3, should not exceed 0.85 per cent. No doubt modifi-cations based on delicate colorimetric determinations of excess iodine can be developed suitable for the 1–10 microgram range, and an an-timony method based on the same principles is anticipated this year.

In view of these new developments, the possible avenues for further work that they open, and the limited time available, it is difficult for the Referee to make proper recommendations for future work. Perhaps a comparative estimation of possibilities will give a clue to the prospects for the greatest ultimate progress.

How appears to have extended the Gutzeit range materially, increased the accuracy to a certain extent, but made the method more complex. No shortening of the time required for a determination can be expected. Its usefulness would probably be greatest in the 0.1–10.0 microgram range, where the other two methods, unless successfully modified, are the weakest. The Klein-Vorhes[3] xanthate extraction process may become established as another method of arsenic isolation. Suggestions have been made that it might be especially useful in the analysis of organic arsenic compounds. The Referee believes that the colorimetric molybdenum blue arsenic determination, after any system of isolation, should be further developed, preferably by photometric methods, to the lower ranges of arsenic, possibly 1–50 micrograms. If it can be applied on the same solution after an iodine titration, it would become particularly useful as a check determination. This would require a change in the phosphate buffer used in the titration method, but such a change, pro-vided it did not introduce an interference, should not be difficult. The iodine titration method as at present developed is remarkable for its speed and accuracy for 5–500 micrograms of arsenic, and if some modi-fication will take care of quantities less than 5 micrograms, this method

⁵ *This Journal*, **22**, 436 (1939).

should be generally applicable. In view of these considerations, the Referee believes it best to determine first whether 0.1–10.0 microgram quantities of arsenic can be analyzed by cerulean-molybdate or iodine methods, and to keep How's modified Gutzeit in reserve until this question is settled. In the meantime, collaborative work on larger quantities of arsenic by the iodine titration method may be started this year. Collaborative studies as reported by Klein and Vorhes on 100–800 microgram quantities by the molybdate method need not be repeated. It is necessary first to ascertain whether the molybdate method will work for small quantities under collaborative conditions.

Since the collaborative work designed to finish the sample preparation phase of the arsenic methods was not conclusive, it must be repeated. The newer methods of final determination should facilitate this work.

The Referee hopes that an iodine titration method for antimony, in the absence of arsenic, will be ready before the next meeting. If this expectation is fulfilled, the next problem is the separation of micro quantities of arsenic and antimony. The Referee has in mind three or four possibilities, one or more of which may materialize during the year.

The action of arsine or stibine on gold or silver solutions was not studied during the year. The Referee believes that this problem is still worth some investigation, particularly for small quantities. The apparatus described by Cassil and Wichmann[5] should allow the development of these sols in small volumes, followed immediately by photometric measurement. The general and associate referees would welcome the assistance of interested analysts in this development work.

COPPER

The Referee's previous recommendations were to the effect that the colorimetric carbamate method for the determination of copper be placed on a photometric basis. The elements that may interfere in a copper determination are bismuth, cobalt, and nickel, which can produce carbamates of yellowish shades. The associate referee has obtained the absorption curves of the copper complex as well as those of the interfering metals. The striking feature of these curves is the fact that the absorption of the copper-carbamate complex at all wave lengths is so much greater than that of the others that small contaminations of bismuth, cobalt, or nickel are practically negligible. In biological samples these metals are probably seldom encountered in amounts in excess of 0.1 milligram and, therefore, do not cause serious errors. The Referee desires to point out, however, that there is at present considerable interest in the determination of copper in tomato products, where nickel interference may be more important because of contamination from nickel-containing equipment. It is unknown at present whether manufactured tomato products ever will contain sufficient nickel to cause an appreciable error in the

copper determination without previous separation of the two metals. The associate referee indicates that this can be done, if necessary, by precipitation of copper in acid solution with hydrogen sulfide. This is true, but former Associate Referee Coulson recommended with good reason, that precipitation methods be avoided in the determination of micro quantities of copper. The Referee, therefore, believes that an examination of the principle of competitive complexes with reference to cobalt and nickel might be profitable and help to avoid sulfide precipitations. The associate referee utilized this principle when he found that potassium cyanide inhibited the copper carbamate reaction but not that of bismuth, enabling him to correct for the small color effect of bismuth, and determined copper by difference.

The associate referee recommends iso-amyl acetate as the solvent for the copper-carbamate complex rather than amyl alcohol or the heavier carbon tetrachloride. He finds that the acetate solvent provides better agreement with Beer's law than either of the other two solvents. In addition he desires to avoid the use of separatory funnels.

The associate referee believes that the copper carbamate method with photometric measurement is now ready for use. The Referee is inclined to agree with this view, but natural caution inclines him to put the method to further collaborative test. He, therefore, recommends that the associate referee formulate a precise copper method based on the experience of former Associate Referee Coulson and his own, including simple directions for making color measurements with several types of instruments, and submit it with some samples to collaborators. In this connection the practice of Coulson in sending ashed samples containing known amounts of copper and interfering elements might well be followed. In such samples the interfering elements of special interest would be bismuth, cobalt, and especially nickel. If a satisfactory micro method for copper can be advanced to tentative adoption at the next meeting of this Association, it can be included in the next edition of *Official and Tentative Methods of Analysis, A.O.A.C.*

The associate referee recommends this photometric copper method for 20–50 micrograms. It could undoubtedly be extended to 100 micrograms by merely increasing the volume of the solvent, but how about the lower limit? The experience this Association has had with the determination of other metals indicates that sooner or later a demand will arise for methods that will satisfactorily determine smaller quantities. The next associate referee might, therefore, think about methods capable of determining 5 micrograms of copper or less.

ZINC

The associate referee and his associate tested 28 metals with dithizone and carbamate reagents in 0.02 N hydrochloric acid and 0.02 N am-

monium hydroxide buffered with ammonium citrate. The Referee would have preferred to work at definite hydrogen-ion concentration rather than at definite normalities, because *p*H is more in line with his conception of the principles governing the dithizone system of separations and analysis. Experience with lead determinations indicates that hydrogen-ion concentration has much to do with optimum extraction of a metal, and that losses may occur if metals are extracted from ammoniacal solutions, by dithizone, at too high a *p*H, or if dithizonates in carbon tetrachloride are washed with insufficiently buffered ammonia solutions.

The associate referees propose a method for the determination of zinc based on dithizone separations and carbamate inhibition of certain dithizone reactions and suggest its collaborative trial. Since the copper method the Association is investigating also depends on the formation of a carbamate complex the two associate referees are approaching a common ground. The Referee concurs in the recommendation for collaborative testing of the proposed zinc method. In addition he suggests that the Associate Referee on Zinc devote some time to a study of the photometric determination of zinc. Other referees are finding photometric determinations to be far superior to ordinary colorimetric methods. Fisher and Weyl[6] state that the maximum absorption for zinc dithizonate in carbon tetrachloride is at 538 mμ. The proper color filter or wave length for use in a dithizone zinc determination is therefore already known.

In addition the Referee hopes that the associate referee will be able to present at the next meeting quantitative data concerning the efficiency of the separations and the inhibitions utilized in the proposed method. The Association should know if small quantities of copper, cobalt, nickel, or other metals are ever transferred with lead and zinc back to the aqueous phase in the separation in the method and if so whether interfering metals are quantitatively inhibited by the mixed reagent. These reactions are equilibrium phenomena that are influenced by many factors, and the success of a separation depends upon choosing optimum conditions. The Referee is particularly interested in learning whether the colored carbamates of cobalt, nickel, bismuth, and especially of copper can ever offer an interference in the determination of zinc in instances where they have not previously been completely separated from zinc. The Associate Referee on Copper finds that the copper carbamate complex has a decided absorption at 540 mμ, in iso-amyl acetate, but that the absorption of cobalt, nickel, and bismuth carbamates is very much smaller. Since 538 mμ is the optimum wave length for the absorption of zinc dithizonate in carbon tetrachloride it can be readily seen that efficient separation of these possibly interfering metals from zinc, or the

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[6] *Wiss. Veröffent. Siemens-Werken*, 14, No. 2, 41 (1935).

complete suppression of both their carbamate and dithizone complexes is necessary.

FLUORINE

The Associate Referee on Fluorine assumed new duties during the year, therefore his report is a summary of the present fluorine situation and of what remains to be done. The Referee has nothing to add and recommends that the Association's work on fluorine be continued along the lines indicated by the associate referee.

LEAD

The associate referee has revised paragraphs **30** and **33** of the rapid spray residue lead methods restricted to the determination of lead on apples and pears, adopted as official, first action, last year. The revision was necessitated because it was found last year that sporadic addition of lime sulfur to washing solutions caused abnormally low lead results if hydrochloric acid was used in the acidification of the apple strip solutions. The remedy is the deletion of the choice of hydrochloric or nitric acid and making the use of nitric acid mandatory. The other revisions concern the deletion of the direct electrolytic lead determination on strip solutions and the substitution of an intermediate dithizone extraction. This safety measure is intended to stop the occasional incomplete recovery of lead from solutions containing abnormal amounts of sugars. These changes do not affect the principles of the methods nor the results. Therefore, the collaborative results reported last year are still valid and the Referee sees no reason why this revision of the rapid spray residue methods should not be adopted. No reports of other difficulties with the spray residue methods on apples and pears have been received during the yea r and adoption of the methods (final action) may be recommended.

The associate referee also makes some observations on the determination o f lead in maple products and baking powders where some difficulties have a risen with respect to sample preparation. It is hoped that information may be obtained before the next meeting on the reported loss of lead on ashing maple sirups. The Referee recommends that some collaborative work on methods for lead determination in these products be started this coming year. The determination of lead in oils or fatty foods has not been investigated. The simplification of methods for removing interferences (bismuth, tin, and thallium) in the lead methods needs attention before the preparation of a new edition of *Official and Tentative Methods of Analysis, A.O.A.C.* The Referee hopes that most of the defects in the lead methods may be remedied before the time for revision, but doubts whether there is time available for completing the project.

MERCURY

The associate referee continued his efforts to shorten the sample preparation and isolation procedures of his dithizone mercury method, now

tentative. Partial oxidation of organic matter followed by concentration of mercury by precipitation on a small filter bed of finely divided metallic zinc or coprecipitation with ferric hydroxide promises to shorten appreciably the amount of oxidation and therefore the time required for sample preparation.

The isolation of mercury from other elements, particularly copper, is making progress. The Referee believes that the possible interference of silver and bismuth should be given some attention. These metals are also neighbors of mercury in the dithizone system and may, under some circumstances, be co-extracted and cause trouble in a mercury determination. Silver and bismuth should not be entirely neglected in mercury determinations in foods or biological samples in general, merely because they are rarely found in such materials.

Since the associate referee determines mercury photometrically by measuring the absorption of residual dithizone rather than of mercury dithizonate, he must by all means avoid oxidation of dithizone in his final extraction. The results seem to show that he is succeeding in doing this by the use of hydroxylamine and hydrazine salts. It seems that the photometric mercury method promises greater accuracy than that possessed by the present tentative titrametric method. The associate referee's future program, especially that of collaborative work, is approved, and it is hoped that at the next meeting decided improvements in all parts of the mercury method may be evident.

SELENIUM

The collaborative results for selenium obtained this year by the volumetric thiosulfate method, together with those obtained in former years by the associate referee and his associates, warranted a recommendation for tentative adoption of this method. A slight, though perhaps significant, loss of selenium was noted if samples were hastily digested in an open system, even in the presence of mercury. It seems, therefore, that analysts should be particularly careful not to hurry the sample preparation in the beginning. If later associate referees consider a closed system advisable, it can be included when the status of the method is changed to official. The Referee also approves of the recommendation for tentative adoption of the thiosulfate method for micro quantities of selenium as applied to foods and biological products, in amounts from 5 micrograms upwards. He believes that future work on selenium should be directed towards the lower ranges (1–10 or 20 micrograms) and that this may be done by (1) refinement of the volumetric thiosulfate procedure, (2) utilization of the very sensitive iodine methods for indirect selenium determinations, and (3) colorimetric methods. The Referee suggests that more sensitive titration methods than are available at present might be developed if the iodine produced by the action of selenium dioxide on po-

tassium iodide in acid solution could be separated from selenium by suitable solvents or other means and oxidized to iodate, and the iodine then liberated with potassium iodide and acid and titrated with thiosulfate to a starch-iodine or electrometric end point. An alternative method for the indirect determination of selenium might be developed by photometric determination of the iodine liberated by selenium dioxide from iodides and then dissolved in a suitable solvent, provided these highly colored solutions follow Beer's law. Selenium dissolves in carbon bisulfide, but its solubility in chloroform or carbon tetrachloride is said to be very low or negligible, although it tends to collect at the interface. The color interference of colloidal selenium to iodine dissolved in these solvents should therefore be slight, although a filtration might be necessary.

As a possible basis for a direct colorimetric determination of selenium suitable for photometric determination of small amounts, the Referee suggests (1) the yellow color produced by selenium dissolved in carbon bisulfide; (2) the red color of colloidal selenium, if it can be properly dispersed by some colloid and the particle size maintained constant long enough for a measurement; and (3) the blue or blue-green color of the selenium-codeine complex in sulfuric acid. It is not expected that all of these color suggestions will be suitable, because of a probable lack of the desired accuracy or sensitivity. The criterion to be used in evaluating them should, in the Referee's opinion, be their value in determining small quantities of selenium of the order of 1–10 or possibly 20 micrograms.

FUMIGATION RESIDUES

This is the first year that a report on the determination of fumigation residues in foods has been given in this section. Since the study of the determination of spray residues was perhaps the principal reason for the existence of the section, the inclusion of the determination of fumigation residues might follow logically. The work of the first associate referee was limited to the study of the determination of cyanides.

It was generally thought heretofore that hydrocyanic acid absorbed by foods did not remain to any dangerous extent or for any great length of time, being either volatilized or changed into the stable cyanohydrin-aldehyde complex, which is believed to be of low toxicity. Last year, however, raisins containing dangerous amounts of cyanides as the result of fumigation with liquid hydrocyanic acid were distributed in interstate commerce. The methods used for the determination of the cyanide residues were neither very accurate nor satisfactory and the problem was given to a new associate referee. The associate referee's first report indicates that a change from distillation to aeration from heated solutions, with alkaline silver titration of the isolated hydrocyanic acid, will accom-

plish much towards sharpening the accuracy of the determination. Since samples containing hydrocyanic acid or cyanides are unstable, collaborative samples can not be distributed, but collaborators can determine their own recoveries and in this manner check the associate referee's work. The Referee hopes that a sufficient number of recovery experiments will be made to form the basis for a recommendation for tentative adoption next year. A method for cyanides accurate to .5 p.p.m. should be satisfactory for all ordinary purposes. If greater sensitivity is demanded, the associate referee has two colorimetric methods of considerable promise in reserve for development. Therefore the Referee recommends that the project be continued.

RECOMMENDATIONS OF THE REFEREE[1]

It is recommended—

(1) That studies be continued on methods of sample preparation of products containing organic arsenic or substances that inhibit the evolution of arsine.

(2) That the arsine evolution-iodine titration method for the determination of 5–500 micrograms of arsenic be studied collaboratively, and that a similar method for antimony and a colorimetric-iodine method for 1–20 micrograms of arsenic be investigated.

(3) That colorimetric methods for arsenic, after adequate systems of isolation, be studied photometrically. The molybdenum blue method for arsenic and gold or silver sol formation by arsine or stibine are especially recommended for attention.

(4) That separation of micro quantities of arsenic and antimony where they occur simultaneously be studied.

(5) That studies on micro methods for the determination of copper be continued.

(6) That the study of methods for the determination of micro amounts of fluorine in foods be continued and that special attention be given to sample preparation of organic materials.

(7) That the revision of paragraphs 30 and 33 of the rapid lead methods restricted to the determination of lead on apples and pears recommended by the associate referee be adopted and that the revised method be made official (final action).

(8) That studies concerned with the determination of lead in oils, baking powders, and maple sirup and with simplification of methods for separation of the interfering metals, bismuth, tin, and thallium, be continued.

(9) That studies on mercury methods be continued.

(10) That the thiosulfate selenium method described by the associate referee last year and subjected to collaboration this year be adopted as

tentative for the determination of selenium in foods in amounts from 5 micrograms upward and that studies be continued on methods especially designed for 1–20 micrograms of selenium.

(11) That the study of micro methods for the determination of zinc be continued.

(12) That checks on the recovery of cyanides added to foods by the aeration-titration method be made and that development of the two colorimetric methods for the determination of cyanides described by the associate referee be continued.

REPORT ON ARSENIC

By C. C. CASSIL (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*

The collaborative studies made during 1938 on sample preparation, as proposed by the Associate Referee in 1937, *This Journal*, **20**, 171 (1937), were not satisfactory. Composite samples of shrimp and tobacco were sent to six collaborators. Each analyst was given instructions to prepare the samples by a wet sulfuric-nitric-perchloric acid digestion and also by a dry ashing procedure. It was further specified that the official Gutzeit method be used for the final determination.

The collaborators obtained results varying from 4 to 20 micrograms of arsenious oxide per gram of shrimp and from 10 to 51 micrograms of arsenious oxide per gram of tobacco, whereas the Associate Referee and two collaborators working on the same samples the previous year found 20–21.5 and 30–31.5 micrograms for the shrimp and tobacco, respectively. The Associate Referee believes that such erratic results are due to variations in the Gutzeit procedure and not to the sample preparation.

It is suggested that collaborative work on sample preparation for arsenic be dropped until there is available an arsenic method that has a higher degree of accuracy and precision than the present official method. Collaborative work on the new method mentioned below will be done during 1939, and if it proves satisfactory it is hoped that the sample preparation studies can be completed in 1940.

A method for determining from 5 to 500 micrograms of arsenious oxide that requires approximately only 10 minutes has been developed. An average recovery of 99.5 per cent with a standard deviation of 0.85 per cent was obtained from 34 determinations. This procedure is described in detail in a paper, entitled "A Rapid Volumetric Micro Method for Arsenic," by C. C. Cassil and H. J. Wichmann in *This Journal* (see p. 436). Some preliminary experiments show that this procedure may also be adapted to the determination of antimony, and that it may be possible to publish a paper during the coming year on antimony and the separation of antimony and arsenic.

RECOMMENDATIONS[1]

It is recommended—

(1) That the rapid volumetric method for quantities of arsenious oxide between 5 and 500 micrograms be studied by collaborators.

(2) That further work be done by the Associate Referee toward adapting this rapid isolation method to quantities of arsenic less than the equivalent of 5 micrograms of arsenious oxide.

(3) That some attention be given to the molybdenum blue method for determining quantities of arsenic less than the equivalent of 50 micrograms of arsenious oxide.

REPORT ON COPPER

By DAVID L. DRABKIN (Department of Physiological Chemistry, Medical School, University of Pennsylvania, Philadelphia, Pa.), *Associate Referee*

In this report an improved method for the determination of small amounts of copper by means of the sodium diethyl dithiocarbamate reagent is presented.

Improvement in technic and increased reliability have been accomplished by appropriate changes in (a) the ashing of the sample and treatment of the ash, (b) the use of a new solvent, iso-amyl acetate, for extraction of the colored copper carbamate complex, and (c) the use of several monochromatic light filters for more precise photometry of the colored solution. The interference of nickel, cobalt, and bismuth, when present in sufficient quantities to be significant, has also been studied.

The literature on the carbamate methods does not include precise data on the light transmission properties of the metallic complexes of diethyl dithiocarbamate, nor on suitable light filters. Such data, obtained both by visual spectrophotometry and by a photoelectric filter photometer, are presented. The choice of solvent and of light filters, and the decision as to the optimal concentrations of copper suitable for photometric measurement are based upon the spectrophotometric findings.

ASHING OF THE SAMPLE

Proper care must be exercised in the ashing of samples, both from the standpoint of possible adventitious contamination with copper as well as losses of the metal (in the form of volatile salts), when the ashing is at too high a temperature. The size of the sample should be such as to provide 20–50 micrograms of copper for analysis. A simultaneous determination should be run on a sample that has been enriched by at least 50 per cent by the addition of standard copper solution, providing a recovery check. Different materials require appropriate modifications in the ashing procedure. Several examples follow:

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).

Milk.—A 200 cc. sample is placed into a large evaporating dish (300 cc. capacity), of silica or platinum, preferably the latter. The sample is heated to a temperature of about 40° C., and while being gently stirred, dilute HCl is added dropwise until isoelectric precipitation of the casein is effected. This procedure is necessary to prevent frothing in the subsequent evaporation of the whey and curd. Evaporation to a caramelized dry mass is carried out upon an electric hot plate, covered with a layer of sand (to insure greater constancy of temperature). During this procedure the evaporating dish is kept covered with an inverted wide-stemmed glass powder funnel of suitable size (approximately 150 mm. diameter). The evaporating dish containing the relatively dry sample is now transferred uncovered to a muffle furnace preheated to scarcely perceptible dull red heat (500°–550° C.). The door of the furnace is kept open until incineration is over. The door is then closed and ashing accomplished.

Elixirs.—Sirupy material, containing a relatively large amount of carbohydrate and aromatic substances, is best ashed by a combination of wet and dry ashing. Partial wet ashing is carried out by successive additions to the sample of small amounts of concentrated nitric acid, with evaporation after each addition upon an electric hot plate covered with a layer of sand. The best commercial grades of C.P. nitric acid, sp. gr. 1.42, should be redistilled in glass at a temperature of 120° C., thereby rendering the acid practically copper free. After partial wet ashing, the sample is completely dry ashed in a muffle furnace at 500° C.

Animal tissues.—In metabolism experiments, if possible the tissues should be relatively free of blood. To obtain such tissue samples the animal may be slowly exanguinated, while the circulatory system is perfused with 0.9 per cent sodium chloride. Appropriately sized tissue samples are then withdrawn, washed quickly with copper-free distilled water, blotted upon filter paper, and quickly weighed in silica dishes of approximately 50 cc. capacity. The samples are then dried by heating in an oven at 110° C. for 24 hours, and re-weighed to furnish the dry weight. In the case of tissues relatively rich in copper, 1–5 grams of wet tissue suffices; in the case of other tissues as much as 20 grams is needed. One to 2 grams of liver, 5 grams of kidney, 20 grams of spleen, and 20 cc. of blood are examples of the size of sample usually taken for analysis. The dried tissue samples may be ashed by a combination of wet and dry ashing as described previously. A more rapid procedure is the following: The sample is partially wet ashed with nitric acid and heated to dryness upon an electric hot plate. The sample is cooled, and 5 cc. of an oxidation mixture, composed of 20 per cent magnesium nitrate saturated with magnesium carbonate is added. The material is ignited and ashed. The ash is taken up with dilute hydrochloric acid and filtered. This is a slight modification of the procedure recently recommended by van Niekerk

(1). Copper checks should be run upon the oxidation mixture, or the standard solutions should also be exposed to the oxidation procedure.

ISO-AMYL ACETATE AS SOLVENT FOR THE COPPER DIETHYL DITHIOCARBAMATE COMPLEX

Since metallic complexes with such reagents as ethyl xanthate, diphenylthiocarbazone, and diethyl dithiocarbamate are but sparingly soluble in water, various non-polar organic solvents have been suggested for their extraction. In the case of copper diethyl dithiocarbamate, McFarlane (2) has suggested the use of iso-amyl alcohol, while Haddock and Evers (3) used carbon tetrachloride. The latter solvent has the advantage of far lower solubility in water than the former. Carbon tetrachloride is, however, more volatile than iso-amyl alcohol, and, being heavier than water, extraction must be carried out in separatory funnels. To avoid contamination with stopcock grease, the separatory funnels must be used with ungreased stopcocks, which is relatively troublesome.

The writer has found iso-amyl acetate to possess decided advantages over both above solvents for the extraction of copper diethyl dithiocarbamate from weakly ammoniacal solutions (*p*H 8.5–9.0). In comparison with iso-amyl alcohol, iso-amyl acetate has a slightly higher boiling point (139° against 130.5° C.) and about one-twentieth the solubility in water at room temperature (0.16 per cent at 25° C. against 3.3 per cent at 22° C.). The latter property no doubt accounts for a far more efficient and rapid stratification of the aqueous (below) and organic solvent (above) layers after thorough mixing. The recovery of iso-amyl acetate is appreciably more quantitative than that of iso-amyl alcohol under the conditions used, which may be partly responsible for the superior photometric results (in respect to conformity with Beer's law) which have been obtained.

It is well recognized that commercial, C.P. grades of solvents which go under the name of "amyl acetate" are largely iso-amyl acetate. The C.P. grade of amyl acetate should be fractionated by distillation. The distillate obtained below 136° C. (sometimes as much as 60 per cent of the material) is discarded. The distillate obtained between 136° and 140° C. is used. This is the solvent referred to as iso-amyl acetate.

Before the data presented in the figures are described, a brief explanation of Beer's law (4) and the term extinction coefficient, ϵ, fundamental in photometry, seems appropriate. Beer's law may be formulated as follows: If by transmission through a layer of absorbing solution of depth 1 (as 1 cm.), light of intensity I is reduced to intensity $I \cdot 1/n$, then by passing through two such layers it will be reduced to intensity $I \cdot 1/n \cdot 1/n$ or $I \cdot 1/n^2$ and, by passing through d such layers, the resulting intensity will be $I \cdot 1/n^d$. Thus as the depth of absorbing stratum increases in arithmetic ratio, the intensity of the light passing through decreases

exponentially or logarithmically. A general equation, expressing Beer's law, may be written—

$$I' = I \cdot \frac{1}{n^d},$$

(1)

where I equals the intensity of incident light, and I' equals the intensity of the transmitted light, after it has traversed an absorbing solution of some finite depth, d. This equation may be written in logarithmic form

$$\log n = -\frac{\log \dfrac{I'}{I}}{d}.$$

(2)

It is not necessary to develop the equation further here, except to say that when a spectrophotometer is employed the measurement which is obtained is that of fraction of light transmitted, T, which is equal to I'/I. Since the chemist is interested in the absorption of light and not primarily the transmission, the extinction coefficient, ϵ, is calculated from the transmission measurement. By Bunsen and Roscoe's definition (5), ϵ is equal to $\log n$ in equation 2, and the equation may be written—

$$\epsilon = -\frac{\log T}{d}.$$

(3)

Since the depth, d, is referred commonly to unity, as 1 cm., the equation becomes

$$\epsilon = -\log T.$$

(4)

In the Bausch and Lomb spectrophotometer, T is equal to $\tan^2 \theta$, the angle at which solvent and solution match (6). Thus, Beer's law is implicit in the equation for the extinction coefficient, and ϵ is directly proportional to the concentration when Beer's law applies. It must be remembered, however, that Beer's law holds strictly true only for monochromatic light. It is this consideration that calls for the use of monochromatic light filters when photometric measurements are carried out with comparators of the Duboscq "colorimeter" type, or with the usual type of photoelectric comparator.

Figure 1 includes data obtained by means of precise, visual spectrophotometry in which a Bausch and Lomb spectrophotometer was used, and similar data obtained by means of a photoelectric filter photometer, the Evelyn apparatus (7). The amounts of copper exposed to extraction by 10 cc. of solvent are given in the abscissa. The values of ϵ appear in the ordinate. The subscript and superscript attached to ϵ denote that the value ϵ was in all cases calculated for the same concentration, $c = 0.1$ mg. per 10 cc. and for the same depth, 1 cm. The depth of cuvette employed in the spectrophotometer was exactly 1 cm., the optical ends

of the cuvette being polished, parallel glass end-plates. In the Evelyn apparatus matched test tubes are employed as cuvettes. The internal diameter of the test tubes was 1.98 cm. The ϵ values in this case were divided by 1.98 (see Equation 3) to obtain ϵ for a depth of 1 cm. This correction is, of course, a very rough one, since the effective optical depth of an imperfect cylinder, only part of which is exposed to the passage of light (as in the Evelyn apparatus) is very difficult to determine
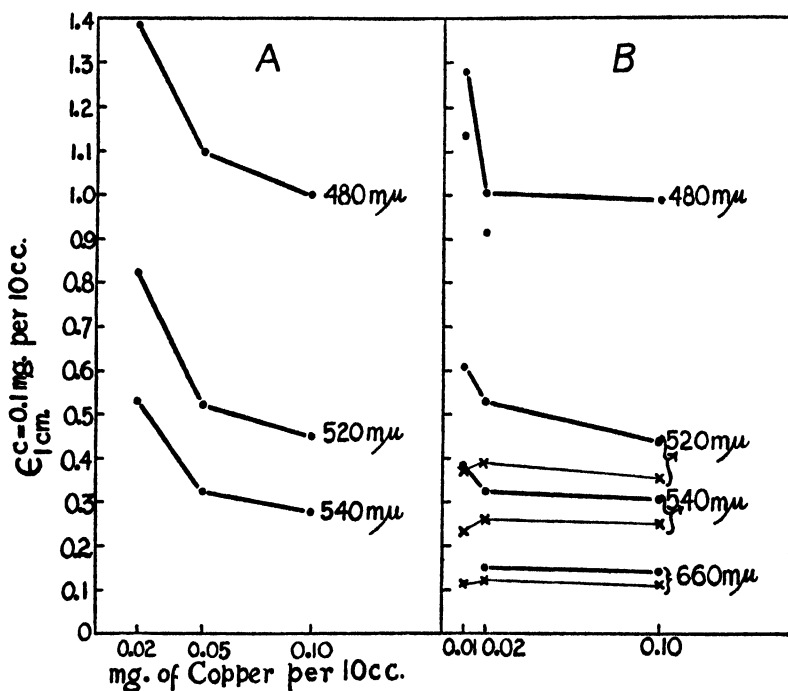
FIG. 1.—COMPARISON OF EXTINCTION COEFFICIENT VALUES, ϵ, YIELDED AT DIFFERENT WAVE-LENGTHS BY THE COMPLEX OF COPPER DIETHYL DITHIOCARBAMATE IN A, ISO-AMYL ALCOHOL, AND IN B, ISO-AMYL ACETATE.

Heavy lines show data obtained by precise, visual spectrophotometry (Bausch and Lomb spectrophotometer), while light lines give data obtained by means of photoelectric filter photometry (Evelyn apparatus).

The amount of copper contained in 10 cc. of the solvent in a particular determination is given in the abscissa. The ϵ values, however, have all been calculated for a concentration of 0.1 mg. per 10 cc. and for a cuvette depth of 1 cm. When Beer's law is obeyed strictly, this manner of plotting data should produce lines parallel with the horizontal ordinate.

precisely. For this as well as other reasons (use of filters with broad spectral intervals instead of true monochromacy attainable in the spectrophotometer), ϵ values obtained with filter photometers are not expected to duplicate the ϵ values obtained by spectrophotometry, which may be considered to be the ultimate method in determinations of this type. With solutions of the type under discussion, however, there is a good parallelism of results obtained by the two methods of measurement. When

results are plotted as in Figure 1, perfect agreement with Beer's law should produce straight lines parallel with the abscissa. It is evident that with iso-amyl alcohol satisfactory agreement with the law (proportionality of ϵ values and concentration) was not obtained, whereas fair agreement was found when iso-amyl acetate was used, particularly in concentrations between 0.02 mg. and 0.1 mg. per 10 cc. solvent used for extraction.

In Figure 2 are shown the absorption spectra of $CuSO_4 \cdot 4NH_3 \cdot H_2O$, Cu diethyl dithiocarbamate, Ni diethyl dithiocarbamate, Co diethyl dithiocarbamate, and Bi diethyl dithiocarbamate. The original copper salt used was prepared from Bureau of Standards Cu, as described by Coulson (8). C.P. nickel chloride and C.P. cobalt chloride were dissolved in water, while bismuth nitrate was dissolved in dilute nitric acid. The carbamate complexes of these various metals were obtained as in the procedure for copper described by Coulson (8). The carbamate metallic complexes were all extracted by means of iso-amyl acetate from the weakly ammoniacal solutions at pH 8.5–9.0. The curves are based upon measurements with the Bausch and Lomb spectrophotometer. The extinction coefficient values are calculated for a concentration of 1 gram molecular weight of metal per liter, at a depth of 1 cm. ϵ values, under such conditions, are often referred to as molecular extinction coefficients. The legend of the figure gives the concentrations employed in the actual measurements. The curve for cupric ammonium sulfate is based on the results of Drabkin and Austin (6) and is given for the interesting comparison it affords as to the relative amount of absorption produced by this copper complex, and that produced by copper diethyl dithiocarbamate. As the figure shows, the relative optical densities are such that the curves had to be drawn against different ordinates. Copper diethyl dithiocarbamate is a far more intense pigment, having approximately 30 times greater absorption in the red-yellow, 100 times greater absorption in the green, and 13,000 times greater absorption in the blue-violet spectral regions than cupric ammonium sulfate.

The two absorption curves for the copper diethyl dithiocarbamate complex were obtained with the respective concentrations of 0.1 mg. and 0.02 mg. copper per 10 cc. of solvent used for extraction. Since the ϵ values are calculated to a common concentration, the two curves should be practically identical within the limits of the spectrophotometric method, provided Beer's law is obeyed. It is evident that fair agreement was attained at all wave-lengths except in the blue-violet. In this region (470–420 mμ) of the spectrum agreement would have been accidental, since the sensitivity of the eye is very poor for light of these wave-lengths. It is to be noted that there is an absorption maximum at wave-length 440 mμ. Ordinarily, a region of maximum of absorption is an ideal place in the spectrum to choose for establishing absorption constants. It is

usually also the best region to isolate by means of monochromatic light filters when measurements are to be carried out by means of a filter photometer. In the present instance, however, the absorption maximum lies beyond the region of ordinary visual sensitivity and is not suitable for accurate visual measurements. Under such circumstances, the next
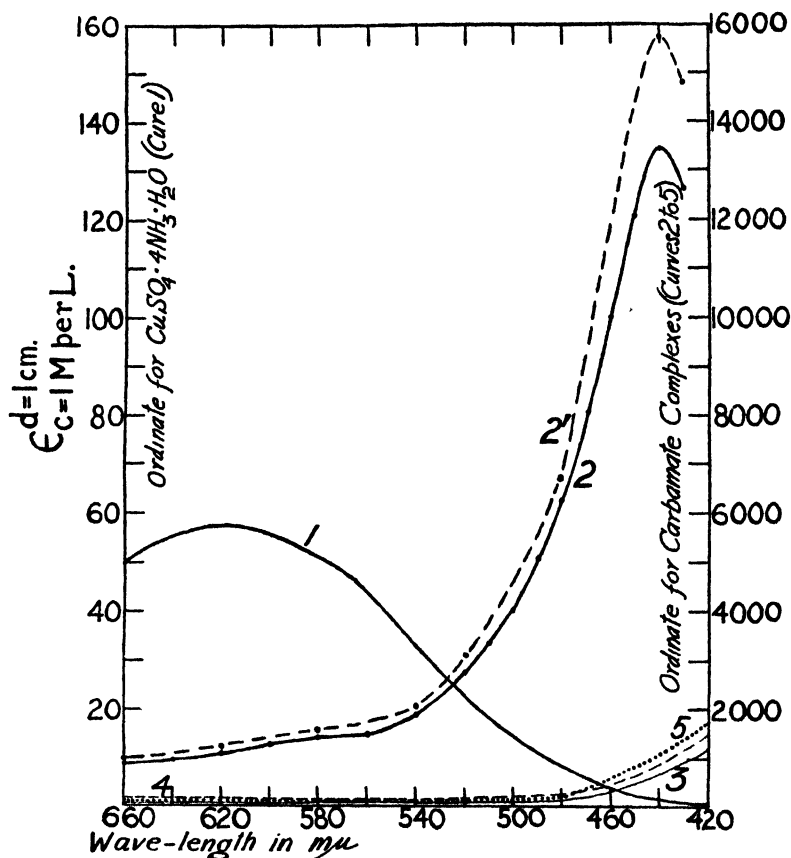
FIG. 2.—ABSORPTION SPECTRA OF METALLIC COMPLEXES OF COPPER, NICKEL, COBALT AND BISMUTH, IN ISO-AMYL ACETATE, OBTAINED BY PRECISE, VISUAL SPECTRO-PHOTOMETRY (BAUSCH AND LOMB SPECTROPHOTOMETER). THE MOLECULAR EXTINC-TION COEFFICIENT (I.E. ϵ FOR A CONCENTRATION OF 1 GRAM MOLE OF METAL PER LITER AND FOR A CUVETTE DEPTH OF 1 CM.) IS PLOTTED AGAINST WAVE-LENGTH. THE ORDINATE ON THE LEFT GIVES VALUES WHICH APPLY ONLY TO CURVE 1, WHILE THE ORDINATE ON THE RIGHT IS APPLICABLE TO CURVES 2 TO 5.

Curve 1. The molecular extinction coefficient curve of $CuSO_4.4NH_3.H_2O$.
Curve 2. The molecular extinction coefficient curve of copper diethyl dithiocarbamate in iso-amyl acetate. The concentration used in the determination was 0.1 mg. of Cu extracted by 10 cc. of solvent.
Curve 2'. Same as curve 2, but determinations were upon a solution containing 0.02 mg. of Cu extracted by 10 cc. of solvent.
Curve 3. The molecular extinction coefficient curve of nickel diethyl dithiocarbamate in iso-amyl acetate. The concentration used in the determination was 0.1 mg. of Ni extracted by 10 cc. of solvent.
Curve 4. The molecular extinction coefficient curve of cobalt diethyl dithiocarbamate in iso-amyl acetate. The concentration used in the determination was 0.1 mg. of Co extracted by 10 cc. of solvent.
Curve 5. The molecular extinction coefficient curve of bismuth diethyl dithiocarbamate in iso-amyl acetate. The concentration used in the determination was 0.1 mg. of Bi extracted by 10 cc. of solvent.

best spectral region for monochromatic isolation would be closer to the region of maximum visual acuity and one in which the absorption of the pigment being measured was not changing with respect to wave-length too steeply. Such a region, for copper diethyl dithiocarbamate is, for example, at wave-length 540 mμ. The best practice, however, for filter photometry of pigments of this type is the use of several monochromats, permitting measurements in at least three broad spectral regions, for example, red, green and blue. Filters which may be employed for this purpose will be described below.

The absorption spectra of nickel, cobalt, and bismuth diethyl dithiocarbamates were determined because, under the present conditions of ashing, solution of the ash, and conversion into carbamates, these metals may possibly interfere with the accurate determination of copper. The color of the nickel and bismuth complexes may be described as approximately similar to that of the copper carbamate complex, though perhaps slightly yellower. The color of the cobalt complex is greenish in comparison with the others. The difference in tint is probably accounted for by a minimum in absorption in the case of cobalt diethyl dithiocarbamate in the green spectral region at wave-length 540 mμ. This is difficult to see in the plotted curve, due to the coordinates employed. Under the conditions used it is probable that only a fraction of the bismuth finds its way into the iso-amyl acetate, since extraction is at pH 8.5–9.0. The absorption of the copper complex as Figure 2 indicates, is approximately 20–30 times greater than that of the other metals. This means, for example, that a sample of 0.05 mg. copper, contaminated with 0.025 mg. of nickel, cobalt, or bismuth, would give an increase in absorption (in comparison with pure copper) amounting to 2.5 per cent. Since ϵ is proportional to the concentration, the determination would yield results 2.5 per cent too high, or 0.0513 mg. copper. In these circumstances any correction for contamination by nickel, cobalt, or bismuth may well be neglected. This has been confirmed by subjecting mixtures of copper and the other metals to analysis. When contamination of copper with appreciably more significant amounts of nickel, cobalt or bismuth is suspected, appropriate separation of the metals or corrections must be employed.

In Figure 3 are presented absorption curves of copper diethyl dithiocarbamate obtained by means of the Evelyn photoelectric filter photometer. The concentrations of copper exposed to extraction by 10 cc. of iso-amyl acetate varied from 0.01 mg. to 0.40 mg. It is to be noted that fair agreement with Beer's law was obtained in the red and green spectral regions (wave-length 660 mμ–520 mμ), but not in the blue-violet region. In the Evelyn apparatus, the photoelectric current is measured by means of a galvanometer, with a scale giving transmission, T, directly in units from 0 to 100 per cent. The instrument employs a so-called barrier-layer photocell, which generates a current as light strikes the sensitive surface,

without need for an external e.m.f. Instruments of this type work best under conditions of relatively high luminous flux, and therefore call for the use of relatively dilute solutions, if a battery operated light source
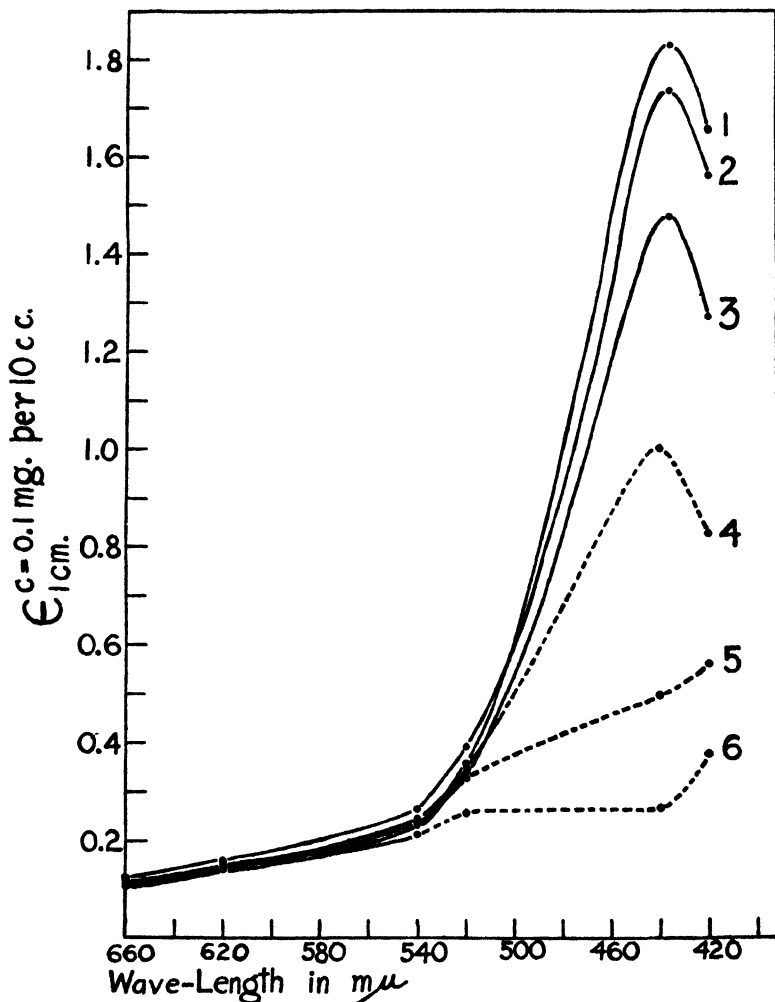
FIG. 3.—ABSORPTION CURVES YIELDED BY THE COMPLEX OF COPPER DIETHYL DITHIOCARBAMATE IN ISO-AMYL ACETATE.

The determinations of transmission were made by means of an Evelyn photoelectric filter photometer in six relatively broad spectral regions, isolated by means of monochromatic filters. The maximum transmissions of the respective monochromats were at wave-lengths 660 mμ, 620 mμ, 545 mμ, 520 mμ, 440 mμ, and 420 mμ. The solid lines were drawn as smooth curves joining dots representing the individual determinations. The broken lines are used to denote uncertainty, due to reading at very low transmission (see text). The concentration of copper in 10 cc. of solvent was

Curve 1,	0.01 mg.
Curve 2,	0.02 mg.
Curve 3,	0.04 mg.
Curve 4,	0.10 mg.
Curve 5,	0.20 mg.
Curve 6,	0.40 mg.

All results, however, have been plotted in terms of ϵ for a concentration of 0.1 mg. of copper per 10 cc. and for a cuvette depth of 1 cm.

of relatively low intensity (6v. flashlight Mazda) is employed. With concentrations of 0.1 mg.–0.4 mg. of copper complex in 10 cc. of solvent, and a test tube cuvette with internal diameter of 1.98 cm., the transmission was too low in the blue spectral region to be determined accurately in an apparatus of this type. The data obtained under these conditions are shown by broken lines. This is an instrumental error, and is not to be taken as indicating necessarily serious deviation from Beer's law in the blue spectral region with higher concentrations of copper diethyl dithiocarbamate. It is possible, however, that such deviations may exist. With concentrations of copper of the order of 0.02–0.05 mg. per 10 cc., excellent reproducibility of determinations was obtained by means of the Evelyn instrument, as shown in Table 1. These results

TABLE 1.—ϵ *values obtained by means of a photoelectric filter photometer upon copper diethyl dithiocarbamate solutions*

Wave-length	SOLUTIONS				Average
$m\mu$	A	B	C	D	
	ϵ^*	ϵ^*	ϵ^*	ϵ^*	ϵ^*
660	0.122	0 125	0 110	0 120	0.119 ± 0.005
545	0.263	0.267	0.260	0.265	0.264 ± 0.002
520	0.394	0.399	0.405	0.395	0.398 ± 0 004
420	1.543	1.560	1.650	1 545	1.575 ± 0.038

* ϵ calculated for concentration of 0.1 mg. copper per 10 cc of iso-amyl acetate used for extraction. Original amount of copper in each sample was 0 02 mg.

may be compared by assuming solution A to be the standard, containing 0.02 mg. copper per 10 cc. The concentration of the other solutions may then be obtained by a ratio of the ϵ values of standard and unknown at each wave-length. For example, at wave-length 660 mμ, 0.02 mg.×0.125 /0.122 = 0.0205 mg., the concentration of solution B. This calculation is carried out at each of the 4 wave-lengths, and the values obtained are averaged. When this is done, in comparison with standard $A = 0.02$ mg. copper, solution $B = 0.0202$ mg., solution $C = 0.0199$ mg., and solution $D = 0.0200$ mg. This is a far greater order of accuracy than has been attained heretofore (8) by non-photometric determinations of copper diethyl dithiocarbamate. Of course, this order of accuracy need not be expected on samples of material which have to pass through an ashing procedure and which may be contaminated with interfering impurities.

Figure 4 presents the transmission curves of various light filters or combinations of filters, which may be used to attain relative monochromacy. The data were obtained with the Bausch and Lomb spectrophotometer. The use of such filters by the writer in the copper diethyl dithiocarbamate method was mentioned in preliminary reports (8 and 9). For visual photometry an ideal monochromat should transmit light effi-

ciently in only one spectral region as narrow as possible. The transmission curve should indicate a sharp, relatively symmetrical falling off in transmission (i.e. increase in light absorption) on the two sides of maximal transmission. Many simple light filters, for example Wratten filter No. 62 (mercury green) and Wratten filter No. 75η (blue green), have good monochromacy in the green and blue spectral regions, respectively, but also transmit light very efficiently in the deep red (Curve 2′, Figure 4).
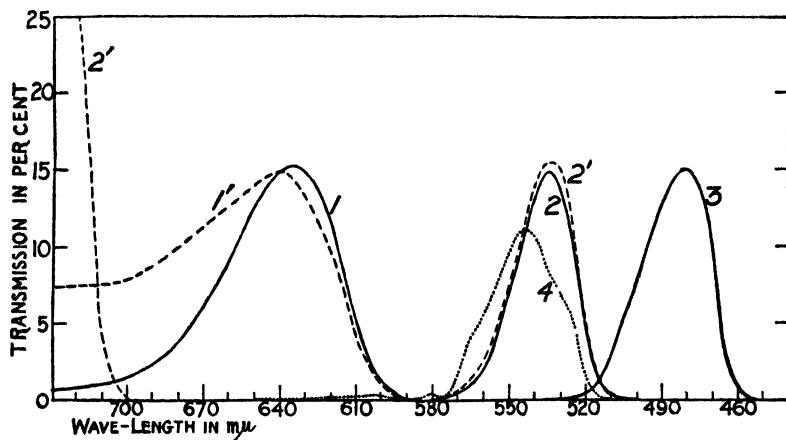
FIG. 4.—THE TRANSMISSION CURVES OF SEVERAL MONOCHROMATIC FILTERS, OBTAINED BY PRECISE, VISUAL SPECTROPHOTOMETRY (BAUSCH AND LOMB SPECTROPHOTOMETER).

Curve 1. The transmission curve of Wratten filter No. 29 F (red for additive synthesis) plus a 1 cm. layer of 0.2805 M CuSO₄ (7.0 grams of crystalline CuSO₄·5H₂O made up to 100 cc.).
Curve 1′. The transmission curve of Wratten filter No. 29 F plus Corning glass filter No. 430 (dark blue green), 0.95 mm. in thickness.
Curve 2. The transmission curve of Wratten filter No. 62 (mercury green) plus a 1 cm. layer of 0.2805 M CuSO₄.
Curve 2′. The transmission curve of Wratten filter No. 62, same as above, but without the liquid CuSO₄ filter. The high transmission in the deep red, which the CuSO₄ eliminates, is evident
Curve 3. The transmission curve of Wratten filter No. 75η (blue green) plus a 1 cm. layer of 0.2805 M CuSO₄.
Curve 4. The transmission curve of a composite Corning glass monochromat of three elements, No. 430 (dark shade blue green), 5.5 mm. thick, No. 351, 2 mm. thick, and No. 512 (didymium glass), 2 mm. thick. This is an example of one of the composite monochromats used in the Evelyn photoelectric photometer.

While such filters may be used unmodified, true monochromacy will not be attained unless means are provided to cut out the red and infra-red transmission. This is particularly desirable if the monochromats are to be employed in photoelectric photometers, since most photocells are very sensitive to the infra-red. The removal of red and infra-red transmission may be accomplished by combining such filters with a liquid filter— a 1 cm. layer of 0.2805 M copper sulfate (7.0 grams of crystalline copper sulfate made up to 100 cc., including a few drops of H₂SO₄ to insure greater stability of the solution). Curve 2, Figure 4, shows the transmission of Wratten filter No. 62, plus a 1 cm. layer of 0.2805 M copper sulfate. A colored glass that exactly duplicates the light transmission

properties of copper sulfate solution is not available. The Corning glass filter No. 430 (dark blue green) very roughly approximates the transmission properties desired. A thickness of 0.95 mm. of the glass may be substituted for a 1 cm. layer of 0.2805 M copper sulfate (Curves 1 and 1′, Figure 4). While this is not ideal, it is simpler practice than the use of liquid filters in appropriate special containers.

Three monochromats, each with approximately 15 per cent relative transmission at their respective minima of absorption, are recommended for use in the photometry of copper diethyl dithiocarbamate. They are:

1. Wratten filter No. 29F (red for additive synthesis), plus Corning glass filter No. 430, 0.95 mm. in thickness. The maximum transmission of this combination is in the red region at wave-length 640 mμ.

2. Wratten filter No. 62 plus the above Corning glass filter. The maximum transmission of the combination is in the green region at wave-length 535 mμ.

3. Wratten filter No. 75η plus the above Corning glass filter. The maximum transmission is in the blue region at wave-length 480 mμ.

By the use of such filters a comparator of the Duboscq type is converted into a filter photometer, and a photoelectric comparator into an abridged spectrophotometer (10). This set of three inexpensive monochromats, which requires only 4 filter elements, will be found useful in a great many other determinations besides that of copper diethyl dithiocarbamate. The Wratten filters should be ordered mounted in B glass. A suitable size, which may be fitted into the eye-piece of a Duboscq instrument, is a 3/4 inch circle. To facilitate determinations upon a solution at each of the three wave-lengths, it may be found convenient to place the Corning glass filter within the eye-piece of the comparator and to mount the three Wratten filters in a simple holder, above the eye-piece, sliding each filter into place as needed. The particular filter combinations that have been discussed are superior in monochromacy and considerably less expensive than corresponding filter combinations of colored glass elements, such as those used in the Evelyn apparatus (Curve 4, Figure 4).

SEPARATION OF COPPER FROM NICKEL AND COBALT AND CORRECTION FOR BISMUTH CONTAMINATION

The most common contaminant of copper in biological materials is iron. Iron forms colored complexes with diethyl dithiocarbamate. Even large quantities of iron (greater than 10 mg.), however, do not interfere with the determination of as little as 0.02 mg. of copper when the copper carbamate complex is extracted in the presence of ammonium citrate at pH 8.5–9.0 (3) and (8). Another effective method for preventing color development due to iron is the use of 4 per cent sodium pyrophosphate, which forms insoluble ferric pyrophosphate, while the soluble copper pyrophosphate is left free to react with diethyl dithiocarbamate. This method for preventing interference by iron in copper determinations,

introduced by Drabkin and Waggoner (11), has been found effective by various workers (1), (2), and (12). The use of pyrophosphate is effective at a pH greater than 7.5 (12), and may possess advantages over the citrate method if it is desirable to work at a pH below 9.0 (as in the presence of excessive phosphates in the material). The advantage of either method is, of course, the avoidance of the necessity for separating copper from iron by means of hydrogen sulfide treatment.

In most biological materials of animal origin, the contamination of copper by nickel, cobalt, or bismuth will be of an order of magnitude that will not produce serious error in the determination of copper as copper diethyl dithiocarbamate, since the development of color due to the other metals will be very small. When considerable contamination by nickel, cobalt, or bismuth is present, neither citrate nor pyrophosphate prevents the formation of the respective carbamate complexes. The only effective means for separating copper from nickel or cobalt appears to be a hydrogen sulfide treatment in acid solution. Under these conditions only the copper forms an insoluble sulfide. An effective method for the formation of cuprous sulfide and its separation is that described by Coulson (13) as Method 1, involving filtration on alundum filtering crucibles and solution in hot nitric acid.

A very effective method has been found by the writer for correcting for contamination of copper by bismuth. One determination is carried out by the usual technic. This yields a value for total pigment as Cu+Bi diethyl dithiocarbamate. In a second determination, after adjustment of the pH but before the addition of diethyl dithiocarbamate, 5 cc. of a 0.08 M solution of potassium cyanide is added (approximately 33 mg. per 5 cc.). The color development obtained in this case is due solely to the bismuth present. The difference between the two analyses represents the color due to copper. It is interesting that nickel and cobalt, besides copper, form very firm complexes with cyanide, probably respectively represented by $Ni(CN)_4^-$, $Co(CN)_6^-$, and $CuCN$, under the conditions employed. Bismuth forms no compound with cyanide.

The Associate Referee believes that with the incorporation of the various improvements in the analytical procedure for copper diethyl dithiocarbamate that have been presented the method may be ready for consideration for acceptance as an official method.

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REPORT ON ZINC*

By E. B. HOLLAND and W. S. RITCHIE (Massachusetts Agricultural Experimental Station, Amherst, Massachusetts), *Associate Referee*

Work on the colorimetric method for the determination of zinc in foodstuffs was continued along essentially the same lines as previously reported. The process has been modified in numerous details and expanded to permit the determination of both copper and lead when desired.

REAGENTS

The purification and preparation of the reagents required more attention than would be expected. The presence of reacting metals in the distilled water, hydrochloric acid, and ammonium hydroxide necessitated careful distillation. Some lots of citric acid or of ammonium citrate required repeated extractions with dithizone and carbon tetrachloride to eliminate the lead, zinc, and copper. After standing in glass containers these same solutions would again react, probably due to the assimilation of zinc or lead from the glass. Therefore frequent checks are obligatory in the analytical work, although there seemed to be some tendency towards stability with continued use.

Care should be exercised in the daily preparation of the dithizone reagent to insure a uniform product and to protect it from unnecessary exposure, especially to light. Most of the carbon tetrachloride may be recovered by treatment with anhydrous sodium sulfate and distillation.

REACTING METALS

To determine the relative response of various metals to dithizone and carbamate reagents chlorides, mostly of high grade, were secured, and solutions were prepared from amounts calculated to contain 1 mg. of the metal to 500 cc. of solution (0.000002 gram per cc. or 2 p.p.m.). Ten cc. (0.02 mg.) was used for each test in 0.02 N hydrochloric acid and ammonium hydroxide solutions. Five cc. of 0.20 N in 50 cc. gave the desired concentration.

As the reagents were known to react after standing the various portions were shaken out with dithizone and carbon tetrachloride to a green color,

and the solvent was discarded immediately before the addition of the metal so that there could be no question as to the source of any colored complex produced. The insolubility of some of the chlorides, such as bismuth, mercurous, palladous, silver, and thallous, may retard or even prevent a reaction that has been reported by others working under a different pH.

The scheme followed and the results obtained are summarized in the following tables.

TABLE 1.—*Method of treatment*

ACID SOLUTION	DITHIZONE 0.01%	CARBAMATE 0.25%	DITHIZONE AND CARBAMATE
	cc.	*cc.*	*cc.*
Water	34	30	29
Metal	10	10	10
Hydrochloric acid, 0.2 N	5	5	5
Dithizone and carbamate	<1	5	<1 & 5
Carbon tetrachloride	10	10	10
AMMONIACAL SOLUTION			
Water	24	20	19
Metal	10	10	10
Ammonium hydroxide, 0.2 N	5	5	5
Ammonium citrate, 10%	10	10	10
Dithizone and carbamate	<1	5	<1 & 5
Carbon tetrachloride	10	10	10

The results (Table 2) were derived largely from single samples of the various salts, but they appear to be consistent. The colored complexes are yellow or pink. All of the reacting metals so far determined belong to one of three small groups of closely related members in the periodic table as follows:

A	B	C
27 Cobalt	46 Palladium, ous	79 Gold
28 Nickel	47 Silver	80 Mercury, ic
29 Copper, ic & ous	48 Cadmium	81 Thallium, ous
30 Zinc		82 Lead
		83 Bismuth

Under the conditions enumerated only copper and mercury (ic) reacted in an acid solution with dithizone reagent. With palladium (ous) and silver the reaction was slight and may be disregarded.

In an ammoniacal solution cobalt, copper (ic and ous), zinc, silver, cadmium, mercury (ic), lead, and bismuth reacted with dithizone although bismuth was very slow. Gold and thallium (ous) may be disregarded.

TABLE 2.—*Color reaction of the metals*

METALS	DITHIZONE		CARBAMATE		DITHIZONE & CARBAMATE	
	0.02 N HCl	0.02 N NH₄OH	0.02 N HCl	0.02 N NH₄OH	0.02 N HCl	0.02 N NH₄OH
Aluminum	green	green	none	none	green	green
Antimony, ous	green	green	none	none	green	green
Beryllium	green	green	none	none	green	green
Bismuth	green	green¹	none	none	green	green
Cadmium	green	pink	none	none	green	green
Cerium, ous	green	green	none	none	green	green
Chromium	green	green	none	none	green	green
Cobalt	green	pink	light yellow	light yellow	green	green
Copper, ic	pink	yellow	bright yellow	bright yellow	green	green
Copper, ous	pink	yellow	bright yellow	bright yellow	green	green
Gold	green	purplish⁵	none	none	green	green
Iron, ic	green	green	none	none	green	green
Lead	green	pink	none	none	green	green
Manganese	green	green	none	none	green	green
Mercury, ic	yellow²	yellow	none	none	green	green
Mercury, ous	green	green	none	none	green	green
Nickel	green	green	light yellow	light yellow	green	green
Palladium, ous	green³	green	none	none	green	green
Platinum, ic	green	green	none	none	green	green
Silver	green⁴	yellow	none	none	green	green
Thallium, ous	green	green	none	none	green	green
Thorium	green	green	none	none	green	green
Tin, ic	green	green	none	none	green	green
Tin, ous	green	green	none	none	green	green
Uranium	green	green	none	none	green	green
Vanadium	green	green	none	none	green	green
Zinc	green	pink	none	none	green	pink
Zirconium	green	green	none	none	green	green

¹ Bismuth reacted yellow on long standing.
² Mercury tended to fade.
³ Palladium, no positive reaction.
⁴ Silver, a slight yellow reaction, destroyed by additional reagent.
⁵ Gold, no positive reaction

Cobalt, nickel, and copper (ic and ous), reacted with carbamate reagent in both acid and ammoniacal solutions. Copper manifested a strong affinity for carbamate and produced a deeper color than did cobalt and nickel. Carbamate with dithizone inhibited the reaction of all these metals except zinc.

PROCESS OF SEPARATION

Eight metals (excluding bismuth) are shown to readily form colored complexes with dithizone or carbamate reagents in 0.02 N acid or ammo-

niacal solutions. These complexes are used for the separation and for the colorimetric determination of specific metals such as lead and zinc.

As the amount of non-reacting bases and acids in the ash solution of most samples materially exceeds that of the reacting metals a preliminary extraction with dithizone and carbon tetrachloride from a 0.02 N ammonium hydroxide solution, buffered with ammonium citrate, is deemed advisable to prevent possible interference. The ammoniacal aqueous layer is of no value and may be discarded.

The solvent layer containing the reacting metals is drawn off and extracted with 0.02 N hydrochloric acid for the removal of lead and zinc (also cobalt, silver, and cadmium) in solution in the acid aqueous layer as chlorides, leaving the copper (also mercury) in the original solvent. The copper in the solvent layer can be determined if desired.

To the acid aqueous layer is added sufficient ammonium hydroxide to produce a 0.02 N solution. The solution is buffered with ammonium citrate, dithizone is added, and it is then extracted with carbon tetrachloride in the presence of carbamate reagent,* which inhibits the lead and leaves the zinc complex in the solvent layer for determination against a standard solution in a Duboscq colorimeter or other instrument.

If the carbamate reagent is omitted and potassium cyanide is added, zinc can be inhibited in the ammoniacal solution and the lead complex determined in the solvent layer in a colorimeter against a standard solution.

With this preamble the various steps of the detailed method should be easily comprehended. The method follows:

ZINC METHOD IN DETAIL
(All water must be redistilled from glass.)

REAGENTS

(a) *Standard zinc solution.*—Dry reagent Zn (30-mesh or finer), transfer 2 grams to a volumetric flask, and add about 200 cc. of water and gradually a slight excess of HCl. Boil until solution is complete and make to 2000 cc. at 25° C. 1 cc. contains 0.001 gram of Zn.

(b) *Dilute zinc solution.*—To 4 cc. of the standard zinc solution add sufficient water at 25° C. to make 2000 cc. 1 cc. contains 0.000002 gram of Zn.

(c) *Hydrochloric acid.*—0.20 N. Distil the HCl into cold "metal-free" water by allowing the concentrated acid to drip from a separatory funnel into hot H_2SO_4 below the surface and dilute to the required strength.

(d) *Ammonium hydroxide.*—0.20 N. Distil the NH_4OH to about 70° C. into cold "metal-free" water and dilute to the required strength.

(e) *Sodium diethyl dithiocarbamate.*—2.5 grams per 1000 cc.

(f) *Diphenylthiocarbazone (dithizone).*†—Dissolve 0.015 gram of the dithizone in 10 cc. of the NH_4OH (d), crushing the aggregates to facilitate solution, and transfer to a 250 cc. pear-shaped separatory funnel with 90 cc. of water. Shake out with 10 cc. portions of CCl_4 to a green color, discard the solvent layers, and filter the aqueous portion through washed ashless paper. Prepare a fresh solution daily.

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* So far as noted R. H. Caughey was the first to observe the action of carbamate in this connection.
† One cc. of reagent is generally sufficient for 0.01 mg. of reacting metals.

(g) *Ammonium citrate solution.*—225 grams †per 2000 cc.* Dissolve the NH₄ citrate in water, add distilled NH₄OH until sharply alkaline to litmus, and make to volume. Transfer 250 cc. to a 750 cc. pear-shaped separatory funnel, add an excess of dithizone (usually 3, 2, and 1 cc., respectively), and shake out with three 25 cc. portions of CCl₄ to a green color. Discard the solvent layers and filter the aqueous portions through washed ashless paper.

(h) *Potassium cyanide solution.*—10% (50 grams per 500 cc.).

(i) *Carbon tetrachloride.*

NOTE: The reagents and the ash solutions are vitiated by standing in contact with glass for any length of time, probably due to the absorption of zinc or possibly lead. This necessitates frequent preparation of reagents or their purification and the prompt handling of analytical solutions. Purified or synthetic ceresin, paraffin, and other wax mixtures have been suggested as protective coatings for the inside of the reagent bottles, and if found to adhere at laboratory temperature may prevent contamination of the solutions.

<center>PROCEDURE</center>

Transfer 4 grams of finely ground (1 mm.) air-dry material to a flat-bottomed platinum dish and calcine to a white or gray ash in an electric muffle at a temperature below visible redness. Pulverize with an agate pestle and reheat if necessary to destroy carbon particles. Transfer the powdered ash to a 100 cc. volumetric flask with small portions of water and a policeman if needed. Add distilled HCl dropwise until the mixture is faintly acid to litmus and then 20 cc. of the 0.20 N HCl in excess. Heat on a steam bath to insure complete solution of the metals, cool, make to volume, and filter through dry ashless paper.

Pipet 10 cc.† (0.40 gram) of the ash solution into a 250 cc. glass-stoppered separatory funnel (pear-shaped with a short delivery tube), add 20 cc. of water, 7 cc.‡ of the 0.20 N NH₄OH solution, 10 cc. of the NH₄ citrate solution, and sufficient dithizone reagent to impart a yellow color to the solution. Add 10 cc. of CCl₄ and shake out vigorously for at least 2 minutes to extract copper, lead, and zinc (also cobalt, cadmium, and mercury when present). Allow the mixture to separate and draw off the solvent layer into a second separatory funnel. Discard the ammoniacal aqueous portion, which should be slightly yellow and contain the non-reacting bases and acids.

To the carbon tetrachloride layer add 45 cc. of water and 5 cc. of the 0.20 N HCl solution and shake out to isolate the copper, removing the lead and zinc as chlorides in the acid aqueous solution, which should be colorless. Draw off the solvent layer and use for the determination of copper if desired, although a 25 cc. aliquot§ of the original ash solution is preferable for copper and lead.

To the acid aqueous layer, add 19 cc. of water, 15 cc. of the 0.20 N NH₄OH solution, 10 cc. of the NH₄ citrate solution, 5 cc. of the carbamate reagent, and sufficient dithizone in small portions to impart a yellow tint to the solution. Add 10 cc. of CCl₄ and shake out to extract the colored zinc salt. Allow to separate, rinse the delivery tube with a few drops of the solvent layer, and draw off the remainder through a dry ashless filter (to remove traces of moisture) into a weighing bottle and stopper to prevent evaporation.

Compare the color in a Duboscq colorimeter, using micro cups and a green color filter against 5 cc. of the dilute zinc solution treated in exactly the same manner.

$$\% = \frac{sR}{R_1} \times 250 \text{ for } 0.40 \text{ gram (F)};$$

$$= \frac{0.0025R}{R_1}$$

$$\text{p.p.m.} = \frac{25R}{R_1}, \text{ in which}$$

s = grams of the standard used.

R = scale reading at which the standard was set.

R_1 = scale reading of the unknown.

F = factor for converting the aliquot to percentage or p.p.m.

Since a small quantity of zinc (about 2 p.p.m.) will usually be found in the blank after careful purification of the reagents, $\frac{(s+B)R}{R_1}$ should be substituted in the calculation and the blank deducted.

Lead may be determined in a similar manner by adding 10 cc. of potassium cyanide instead of carbamate in the third extraction to inhibit the zinc.

The method presented has given promising results and warrants collaborative work on the part of the Association.[1]

REPORT ON FLUORINE IN FOODS

By DAN DAHLE (U. S. Food and Drug Administration,
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The titration of fluorine with thorium nitrate was studied. Such factors as (1) the amount of indicator used, (2) variations in pH, and (3) the presence of neutral salts like sodium chloride and sodium perchlorate and their effects on the accuracy of the titration were given consideration. The details of this work were published in *This Journal*, 21, 459 and 468. In general, it seems that the thorium nitrate titration can be depended on for great accuracy in the determination of small quantities of fluorine when applied to distillates free from weak acids, sulfates, and phosphates, and containing only moderate amounts of chlorides and perchlorates.

There are indications that during the distillation the glassware contributes minute quantities of fluorine. This in turn seems to be compensated by a slight deficiency in the recovery of the distilled fluorine. For practical purposes these errors do not appreciably influence the accuracy of the determination of fluorine in foods, but they may require study and consideration where very minute quantities of fluorine are concerned.

Titrations of distillates from samples distilled without previous destruction of organic matters were only partly successful, which indicates that a complete absence of organic acids in the distillate is desirable for accurate results on fluorine. Ashing of all food materials prior to the

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).

distillation, therefore, seems to be a necessity if the determination is to be made by titration with thorium nitrate.

The problem that remains to be solved is that of separating small quantities of fluorine from large amounts of organic matter without loss or gain of fluorine. So far, no entirely satisfactory ashing procedure is available. The fluorine fixatives suggested by different experimenters have turned out to be unsatisfactory in one way or another. The main troubles seem to have been: (1) a fluorine content in the fixative itself; (2) poor solubility of the fixative, resulting in lack of proper contact between sample and fixative; and (3) various difficulties in getting proper ashing or in the subsequent distillation of the fluorine.

RECOMMENDATIONS[1]

It is recommended—

(1) That the work on fluorine in foods be continued.

(2) That the procedure for titrating small quantities of fluorine be studied collaboratively.

(3) That the question of preparation prior to the distillation be given further study.

REPORT ON LEAD

By P. A. Clifford (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

SPRAY RESIDUE

For the reasons outlined in the Associate Referee's report of last year, *This Journal*, 21, 218 (1938), it is recommended that nitric acid, exclusively, be used for acidification of the wash solution in the rapid solvent method for the determination of spray residue lead on apples and pears. Hydrochloric acid may be retained in the rinse, if desired, to provide for a rapid Gutzeit arsenic determination upon a further aliquot of the same alkaline wash solution, after acidification with hydrochloric acid. It was likewise found desirable to eliminate the *direct* electrolytic determination of lead upon the acidified wash filtrate. Therefore it is recommended that pars. 30 and 33, *Methods of Analysis*, A.O.A.C., 1935, 391, 393, be amended. The changes were published in *This Journal*, 22, 85 (1939). They do not affect the collaborative work reported last year.

MAPLE SIRUP

The analysis of maple sirup for lead has developed into a major project for regulatory chemists within the past two or three years. Accordingly, a rapid colorimetric dithizone method presented by Perlman[2] is of in-

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).
[2] *Ind. Eng. Chem. Anal. Ed.*, 10, 134 (1938).

terest. This author appears to have surmounted the interferences of zinc and tin in this colorimetric procedure, and his own figures and the results obtained by the Associate Referee on a number of samples indicate that the method yields results comparable in accuracy to those obtained by the ash-electrolytic or mush-electrolytic methods. A rapid method, similar in scope to the colorimetric dithizone method for lead on apples and pears, seems highly desirable for maple sirups, and it is the Associate Referee's plan to study Perlman's procedure collaboratively, perhaps to revise it slightly so as to employ a larger sample and less awkward aliquoting, and to contrast results with those obtained by the ash-electrolytic and mush-electrolytic methods.

BAKING POWDERS

A procedure for the determination of lead in baking powder was proposed last year by the Associate Referee, *This Journal*, 21, 437 (1938). Further work, however, showed that certain combination powders yielded a highly refractory ash, which came into solution only after prolonged digestion with hydrochloric acid. As this rendered the analysis of these powders very troublesome, it was thought best to postpone collaborative study until these difficulties were adjusted. It was found later that treatment of the baking powder ash with hot 30 per cent sodium hydroxide solution, followed by an excess of hydrochloric acid, is very effective in securing a clear ash solution. If any appreciable amount of insoluble material remains after this treatment, the writer's practice has been to transfer the ash solution and residue from the casserole to a centrifuge bottle, clarify, pour off the clear solution, and rinse the residue (chiefly silicates and sulfates) into a platinum dish. The dry residue is then treated with hydrofluoric acid plus a few cc. of sulfuric acid, and usually no trace of insoluble material remains. The dish is then rinsed into the main body of the solution and the analysis continued as usual. This procedure will be studied collaboratively next year.

It might be well at this point to stress the necessity of bringing *all* ash material into solution by suitable means whenever a dry ashing procedure is used in the determination of lead. Appreciable amounts are apt to be occluded in refractory silicate or phosphate residues.[1] The same precaution applies to a calcium sulfate residue after a wet digestion, and here, even small amounts of barium can cause large losses of lead due to the isomorphous nature of lead and barium sulfate. Hydrochloric acid is usually the best solvent for a dry ash, but in some cases perchloric acid has been found very effective.

PERMANENCY OF DITHIZONE SOLUTIONS

The results of a study of the permanency of dithizone solutions were published by the writer in *This Journal*, 21, 695 (1938). Carbon tetra-

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[1] Fairhall, *Am. J. Pub. Health*, 28, 825 (1938).

chloride solutions of dithizone can be preserved almost indefinitely if the solvent is first purified and the solution stored in the cold under dilute sulfur dioxide water. Stabilization of chloroform solutions of dithizone has not been as successful. Recently, W. O. Winkler[1] advises that dilute sulfuric acid has a stabilizing effect upon chloroform solutions of dithizone. To test this, two portions of the same dithizone solution were stored under ordinary laboratory conditions in well-stoppered white glass flasks with a layer of sulfuric acid $(1+100)$ overlaying one portion. Decomposition rates were followed photometrically over a period of 55 days. At the end of that time, the untreated portion had fallen to 83 per cent, and the acid-treated portion had fallen to 87 per cent, of the original strength. Thus an appreciable stabilizing effect was noted. In a subsequent experiment, however, this stabilizing effect could not be reproduced, and while an untreated solution of dithizone in chloroform dropped to only 91 per cent strength over a period of 33 days, a portion of the same solution overlaid with dilute sulfuric acid fell to 73 per cent of the original strength. In fact, dilute acetic acid seemed to stabilize better than the dilute sulfuric acid in this case as photometric readings on a portion of the same solution overlaid with acetic acid $(1+100)$ indicated a strength of 89 per cent at the end of 33 days.

Thus, the value of dilute acids as stabilizers for chloroform solutions of dithizone is problematical, and until better stabilizers are found, the Associate Referee believes stress should be placed on the absolute purity of the solvent and the dithizone with which dithizone solutions are prepared.

DETECTION OF INTERFERENCES IN THE "MIXED-COLOR" DITHIZONE METHOD FOR LEAD

The remarks of the Associate Referee on the photometric detection of the interferences of bismuth, tin, and thallium in the lead method and on the co-determination of lead and bismuth have been expanded into a contributed paper, which will appear in an early issue of *This Journal*.

It is recommended[2] that paragraphs **30** and **33** be revised as suggested and that collaborative work on lead be continued.

REPORT ON MERCURY

By W. O. WINKLER (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The work on mercury this year has dealt with concentration and isolation procedures and with the photometric method of determination. In accordance with last year's report, *This Journal*, **21**, 220 (1938), work

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[1] Private communication.
[2] For report of Subcommittee C and action by the Association, see *This Journal*, **22**, 62 (1939).

was also continued on methods of concentration to avoid oxidation of the sample.

CONCENTRATION PROCEDURES

(*A*) *Zinc.*—The method of concentration by precipitation with powdered zinc, reported last year, was not further investigated to any extent this year. This is largely due to the fact that the Associate Referee was unable to secure powdered zinc of the desired purity. A few experiments with zinc of 40–80 mesh showed that too large a quantity of this material is necessary to remove all the mercury from solution.

If the accuracy desired does not exceed 5–10 micrograms (.005–.01 mg.) powdered zinc may be used. It is probable that the quantity of zinc necessary for a determination can be reduced by a system of filtrations of the mercury solution through the powdered zinc metal. The Associate Referee shall endeavor to do this and also to secure a metal more nearly mercury-free.

(*B*) *Ferric Hydroxide.*—The Associate Referee last year expressed the intention of using ferric hydroxide as a gatherer or concentrating agent for mercury, and the work this year has shown the value of this reagent for this purpose, which is due to its great powers of adsorption. By a very simple procedure it is possible to completely remove mercury from its solution. The procedure follows:

REMOVAL OF MERCURY FROM SOLUTION

Add to the acid solution of mercury (such as the HNO_3 extract of a food sample) sufficient strong NaOH (35–50%) to neutralize most of the acid. Transfer the solution to one or more centrifuge bottles with the aid of a wash bottle. Add to each bottle (150–180 cc. volume) 2 cc. of a nearly saturated solution of Fe $NH_4(SO_4)_2$ and continue the addition of the NaOH slowly with stirring until the solution is basic to litmus. Add 1 cc. of NaOH in excess and continue stirring the liquid for about 1 minute. Place the bottles in a centrifuge and whirl 8–10 minutes at high speed. After decanting the supernatant liquid, immediately add 15 cc. of HNO_3 (1 +5) and allow the bottle to stand with occasional rotary shaking until the $Fe(OH)_3$ is dissolved. Place the covered bottle on the steam bath for 5 minutes. Remove, and add sufficient $KMnO_4$ solution (saturated) to oxidize any organic matter and bring any reduced mercury to the mercuric state. Replace the bottle (covered) on the steam bath and again heat several minutes. (If a considerable quantity of organic matter has been precipitated with the $Fe(OH)_3$ the HNO_3 mixture should be placed in the reflux apparatus used in preparation of the original sample extract and boiled with sufficient $KMnO_4$ to maintain a purple color.)

Remove the bottle from the bath, dilute with 25–30 cc. of water, and reduce the $KMnO_4$ etc., with a 10% solution of oxalic acid. Partially neutralize the excess acid with 3 cc. of NH_4OH (1 +1) and dilute to about 200 cc. with water. Extract the mercury from the solution with dithizone as described in a previous report, *This Journal*, 18, 641 (1935). Use carbon tetrachloride as the solvent for the extractant if the extracts are to be treated according to procedure No. 2.

TREATMENT OF DITHIZONE EXTRACTS

Treat the extracts by one of the following procedures:

(1) Oxidize the extracts directly in the $CHCl_3$ or CCl_4 solution by the modification of the original methods, *This Journal*, 19, 236 (1936), given below.

(2) Transfer the mercury to an aqueous solution by shaking the dithizone extracts with an acid aqueous solution containing thiosulfate and oxidize the thiosulfate by Method 2. See also *Ibid.*, 21, 220 (1938).

If copper is present, remove by the acid iodide treatment given in a previous report, *Ibid.*, 18, 642 (1935), reextract the mercury from the solution after making ammoniacal and follow by procedure No. 2.

(1) DIRECT OXIDATION OF EXTRACTS

Direct oxidation of the extracts is accomplished by shaking them with an acid solution of permanganate that has been warmed to 50°–55° C. The use of nitric acid in the oxidation solution has been substituted for sulfuric acid because of the greater solvent power of nitric acid for mercury. A volume of 1.5 cc. of nitric acid (1+1) is used. Some results by this modified treatment are given in Table 1. The determinations were made photometrically.

TABLE 1.—*Recovery of mercury by direct oxidation of extracts*

MERCURY ADDED	MERCURY FOUND	DIFFERENCE
mmg.	*mmg.*	*mmg.*
10.0	10.0	0
15.0	14.9	0.1
1.0	1.6	0.6
2.0	2.6	0.6
15.0	16.1	1.1
10.0	11.1	1.1

The seemingly high results are thought to be due to the attack of the chloroform solvent by the oxidizing mixture, and further experiment with other procedures indicates that the reducing agent used was not entirely satisfactory. The use of hydroxylamine hydrochloride as the reducing agent, reported last year, has not proved quite so effective as was anticipated. Reduction with hydroxylamine sulfate followed by addition of hydrazine sulfate has been found to maintain the stability of the dithizone very effectively. The fact that the excess mercury recovered is not uniform does not permit the conclusion that it was contributed by the reagents, but rather shows that the dithizone reagent was affected. The results indicate that the procedure is promising.

(2) TRANSFER TO AQUEOUS SOLUTION WITH THIOSULFATE AND OXIDATION OF THE AQUEOUS SOLUTION

The transfer of the mercury from the dithizone extracts to the acid thiosulfate solution appears to be complete. In the procedure given last year, the oxidation following the transfer was accomplished by adding potassium permanganate in the cold. As occasional losses have been encountered by this method of oxidation, the oxidation is now done in the presence of nitric acid and sulfuric acid, accompanied by heating on the steam bath for 10 minutes. Heating the solution to the temperature

of the steam bath makes necessary removal by filtration of any dithizone solvent that may cling to the aqueous solution. The procedure is as follows:

Add to the combined dithizone extracts in a 150 cc. separatory funnel, 45 cc. of water, 2 cc. of H_2SO_4 (1 +50) and 3–4 cc. of 2% $Na_2S_2O_3 \cdot 5H_2O$ in the order given. Shake the funnel vigorously for 30–45 seconds. Allow the layers to separate and immediately draw off the lower CCl_4 layer. Filter the aqueous layer through a small wet pledget of cotton in a short-stemmed funnel and collect the filtrate in a 150 cc. beaker. Wash the separatory funnel and filter with 2 or 3 small portions of water from a wash bottle. Add to the aqueous solution 4 cc. of a 1 to 1 mixture of H_2SO_4 and HNO_3 and then add 5 cc. of saturated $KMnO_4$ solution with stirring. Place the covered beaker on the steam bath for 10 minutes, stirring occasionally. Remove, and cool the solution to room temperature, then neutralize most of the acid with 13 cc. of NH_4OH (1 +1). Reduce the $KMnO_4$ by adding dropwise a 10% $NH_2OH \cdot H_2SO_4$ solution with stirring until a clear solution is obtained. When the solution is clear, add 2 cc. of a saturated solution of $(NH_2)_2 \cdot H_2SO_4$. Make the solution to the proper volume and make the determination.

Results obtained on four samples with this procedure are given in Table 2.

TABLE 2.—*Recovery of mercury by transfer to dilute acid solution with thiosulfate and subsequent oxidation of the thiosulfate*

MERCURY ADDED	MERCURY FOUND	DIFFERENCE
mmg.	*mmg.*	*mmg.*
10.0	10.9	+0.9
0	0.9	+0.9
12.0	12.7	+0.7
14.0	14.6	+0.6

The results (Table 2) are consistently high, which fact indicates that some mercury was contributed by one or more of the reagents. The fact that the readings were constant lends support to the conclusion that the excess mercury found was not due to any instability of the reagent. If the amount of mercury contributed was equal to the average of increases found, then the results obtained are within 0.1–0.2 microgram of the mercury actually present. Further work should demonstrate the possibilities of this procedure.

OXIDATION AND REDUCTION

Before the above method of reducing the oxidant with hydroxylamine sulfate followed by hydrazine sulfate was tried, reduction was made with 30 per cent hydrogen peroxide or hydroxylamine hydrochloride. Provided the oxygen liberated in the reduction is entirely removed the hydrogen peroxide produces a solution with which the dithizone "stays put" very well in the determinative extraction. This is usually accomplished by heating on the hot plate or the steam bath. The main precautions to

avoid loss of mercury in this case are to insure the absence of chlorides, and to heat under a reflux in the presence of excess sulfuric acid. Although the mercuric sulfate is reputedly not volatile in the presence of sulfuric acid occasional losses of mercury have occurred on boiling the solution obtained above in a covered beaker.

Several samples were submitted to one collaborator and the Associate Referee, and the mercury was determined by precipitation with ferric hydroxide followed by the solution in nitric acid, extraction with dithizone, and transfer to the aqueous phase with acid thiosulfate. The sodium thiosulfate was oxidized as previously indicated and excess permanganate reduced with hydrogen peroxide followed by boiling to remove free oxygen and neutralization of most of the acid with ammonium hydroxide (1+1). Results are given in Table 3.

TABLE 3.—*Recovery of mercury by acid thiosulfate transfer followed by oxidation and reduction with hydrogen peroxide*

Hg ADDED	Hg FOUND	DIFFERENCE
mmg.	*mmg.*	*mmg.*
10 0	10.3	+0.3
12 0	12.8	+0.8
14 0	14.0	+0 0
12 0	11.6	−0.4
10.0*	10.2	0.2
12 0*	12 0	0.0
10.0*	9.8	−0.2

* Determinations made by the Associate Referee.

The results (Table 3) obtained on quantities of 10–14 micrograms are within 3–4 per cent of the correct amount in all cases but one. The Associate Referee believes this to be a good average, and the accuracy is sufficient for most purposes where minute quantities of mercury are to be determined.

MANIPULATION OF THE PHOTOMETRIC DETERMINATION

The results shown in Tables 1–3 were obtained by the photometric method, *This Journal*, **21**, 226 (1938), with chloroform solutions of dithizone of 5 and 10 mg. per liter. The Associate Referee prefers a solution of 5 mg. per liter, of which 10 cc. is taken for quantities between 0 and 17.0 micrograms. The appreciable solubility of chloroform in aqueous solutions makes it necessary that the determinative extraction be made from a definite volume and the standards made by extraction from the same volume. The volume of 120 cc. was chosen as convenient for the "thio" transfer potassium permanganate oxidation procedure. The practice was to mark the separatory funnel from which the extraction was to be made at this volume with a glass marking pencil and make the solution

to the mark. Readings were made with a one-inch cell for the solution containing 5 mg. per liter.

Solutions containing 10 mg. per liter were read in a half-inch cell, only 5 cc. of solution being used in most cases. The practice in this case was to saturate the solution to be determined with chloroform before making the determinative extraction, remove the excess chloroform by filtering through a pledget of cotton, and wash with dilute acid solution saturated with chloroform. This last procedure is essential in making the determination following direct oxidation of dithizone in the chloroform solution, in which case the sample solution is already saturated with chloroform.

It has been observed that the mercury dithizone solution does not come to equilibrium in the photometer immediately, and in most cases the reading will be observed to rise. The reading recorded should be at the maximum when equilibrium has been obtained. If the reading starts dropping again while in the photometer, the maximum reading should be used.

RECOMMENDATIONS[1]

It is recommended—

(1) That collaborative work be done on the ferric hydroxide precipitation method of concentration, and that the acid sodium thiosulfate method of isolation given in this report be followed.

(2) That further work be done on the zinc precipitation method.

(3) That work on the photometric method for mercury be continued.

REPORT ON SELENIUM

By R. A. OSBORN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The simplified procedure for the determination of selenium as published in *This Journal*, 21, 228 (1938) was studied collaboratively. The collaborators were given three samples of dry vegetation that had previously been thoroughly ground and mixed, and a standard selenium solution containing 20 micrograms of selenium per milliliter for use as the ultimate standard. The selenium content of each sample was not large. The analysts were directed to digest duplicate 10 gram portions and to titrate with approximately 0.001 N iodine and thiosulfate solutions. Table 1 summarizes the results of the collaborators.

COLLABORATORS

(A) V. H. Morris and Wesley H. Stoneburner, Ohio Agr. Expt. Sta., Wooster, Ohio.

(B) Percy O'Meara, State of Michigan Department of Agriculture, Lansing, Mich., with cooperation of L. H. Greathouse, Michigan State College Experiment Station, Lansing.

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).

(C) K. T. Williams, U. S. Bureau of Chemistry and Soils, Washington, D. C.

(D) H. W. Lakin, U. S. Bureau of Chemistry and Soils, Washington, D. C.

(E) L. H. McRoberts and A. K. Klein, U. S. Food and Drug Administration, San Francisco, Calif.

(F) A. L. Curl, U. S. Food and Drug Administration, Washington, D. C.

(G) N. J. Menard, U. S. Food and Drug Administration, Washington, D. C.

(H) R. A. Osborn, U. S. Food and Drug Administration, Washington, D. C.

TABLE 1.—*Collaborative results*

COLLABORATOR	SAMPLE 1		SAMPLE 2		SAMPLE 3	
	p.p.m.	*Av.*	*p.p.m.*	*Av.*	*p.p.m.*	*Av.*
A	0.36*		12.3*		4.5*	
	0.97		11.2		4.4	
	0.76	0.70	11.7	11.7	4.5	4.45
B	0.14		8.9		3.6	
	0.3		12.4		2.5	
	0.5	0.3	11.4	10.9	2.8	3.0
C			9.6		3.3	
			9.5		3.0	
			9.8	9.6	2.7	3.0
D			5.3		2.7	
			9.6		2.6	
			7.3	7.1	2.4	2.6
E	0.4		9.1		4.5	
	0.4		12.1		3.8	
	0.2	0.3	10.3	10.5	4.9	4.4
F	0.2		12.8		4.0	
	0.2	0.2	13.0	12.9	3.9	3.95
G	0.16		12.7		3.4	
	0.18	0.17	13.0	12.8	3.3	3.35
H	0.20		13.2		3.9	
	0.21		12.7		3.2	
	0.22	0.21	13.3	13.1	4.2	3.8
Grand average		0.35		11.1		3.57

* Regular Robinson still used for the distillation instead of the special still equipped with a thermometer, *Ind. Eng. Chem. Anal. Ed.*, 6, 274–276 (1934).

DISCUSSION

In general the collaborative results are in excellent agreement. Lack of experience in the titrations with 0.001 N solutions may account for variations as large as 1 microgram. It is the experience of the Associate Referee that artificial light is not so desirable as natural daylight for viewing the end point of the titration. It is necessary also to complete the titrations rapidly and to use as nearly as possible the same conditions and amounts of reagents in the titration of the unknowns as are used in

the standardization of the iodine and thiosulfate with the standard selenium solution. Collaborators C and D did not use mercury in the digestion. As it is also the Associate Referee's experience that the mercury functions to reduce volatility of selenium, their lower results may be attributable to its omission.

Additional studies were made of the method of digestion in order to determine whether a closed or trapped system of digestion is better than the open digestion. Eight additional samples of dry vegetation were analyzed. Duplicate 10 gram portions of the samples were digested by (1) complete digestion with the modified Soxhlet apparatus, *This Journal*, **20,**

TABLE 2.—*Further study of digestion procedures*

SAMPLE	DESCRIPTION	PARTS PER MILLION OF SELENIUM		
		PROCEDURE (1)	PROCEDURE (2)	PROCEDURE (3)
5	Broccoli No. 231	285	283	282
		284	282	280
6	Alfalfa No. 234	93	94	92
		93	92	91
7	Vegetation No. 264	105	103	101
		104	103	97
8	Vegetation B 14447	1208	1211	1199
		1205	1208	1198
9	Vegetation No. 122	115 (5. g)	119 (5. g)	115 (5. g)
10	Gluten Flour	1.5	1.6	
		1.3 (5. g)	1.4 (5. g)	—
11	Flour—Plot 6 (1935)	1.4	1.3	
		1.4	1.3	—
12	Wheat Plot 2 (1935)	1.5	1.5	
		1.5	1.4	—

The results in Table 2 were obtained by A L. Curl.

201 (1937), and a very low flame for approximately 3 hours, and the trapped liquid was redistilled; (2) a very slow digestion (about 2 hours) in a Kjeldahl flask until a dark brown color was obtained, *This Journal*, **21,** 234 (1938); (3) as in (2) except a rapid digestion which was discontinued when the mixture became a dark brown color (20–50 minutes). In all instances mercury was used in the digestion and after distillation and precipitation the selenium was measured volumetrically, starch being used as indicator. Samples 5–9 inclusive were titrated with 0.01 N solution, and with Samples 10–12 inclusive 0.001 N solutions were used.

There is excellent agreement between the results with the modified Soxhlet and slow digestion in a Kjeldahl flask. It appears that small

losses of selenium may occur even when mercury is present if the digestion is carried out too rapidly. Incidentally, Sample 5 was also digested by attaching a vertical condenser to the reaction flask and refluxing gently for several hours, the condenser then removed and the digestion continued until SO_3 fumes appeared. The selenium found for 5 gram duplicate portions was 284 and 283 p.p.m. Further study of this procedure may be warranted since it eliminates the trap and appears to give good results

Those carrying out routine selenium determinations may wish to recover the used hydrobromic acid. It is practical to discard all material distilling below 120° C. and to collect and redistil the 120–124° C. fraction. The fraction distilling from 124 to 128° C. need not be redistilled. The Associate Referee uses the recovered acid for the distillation of selenium but the reagent grade acid for the titration, especially in the lower ranges.

Standard selenium solutions are usually stable. Some precipitation of red selenium around the necks of bottles containing standard solutions have been observed, but no material change was apparent in the concentration of the solutions even when they were one or two years old.

It is recommended[1] that the procedure presented by the Associate Referee for the determination of selenium in foods, which has been studied collaboratively, be adopted by the Association as tentative. It is further recommended that studies on the determination of selenium be continued with the view to further simplification or greater precision.

REPORT ON FUMIGATION RESIDUES IN FOODS

By W. O. Winkler (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The Associate Refereeship on Fumigation Residues in Foods was created during the year following the epidemic of cyanided fruits, mainly raisins. As a result it was the interpretation of the Associate Referee that although considerable work might well be directed to the detection of residues from a number of other fumigation materials, for the present all efforts should be directed toward the detection of residual hydrocyanic acid in food products.

In cooperation with the Referee on Metals in Foods an attempt was made to increase the sensitivity and accuracy of the present A.O.A.C. methods, *Methods of Analysis, A.O.A.C.*, 1935, 348, as well as to introduce new methods for qualitative and quantitative determinations. The work has progressed satisfactorily.

In order to increase the sensitivity of the present methods for the determination of hydrocyanic acid in stock feeds, it seemed to be necessary to reduce materially the volume of liquid in which the determination

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is made. The isolation of the hydrocyanic acid by direct or steam distillation gave a volume of liquid too large to permit of real sensitivity, being only about 15–20 p.p.m. It was also frequently found in the case of fruits such as raisins that the distillate possessed an opalescence that further obscured the end point. The Associate Referee prefers the basic titration method because it is simpler and more specific for hydrocyanic acid than the acid precipitation titration method.

Aeration appeared to be the most logical method of isolating and concentrating the hydrocyanic acid, and since the Associate Referee had previously used the Labatti[1] aeration method, a modification of this procedure was tried. The following very satisfactory procedure, combin-
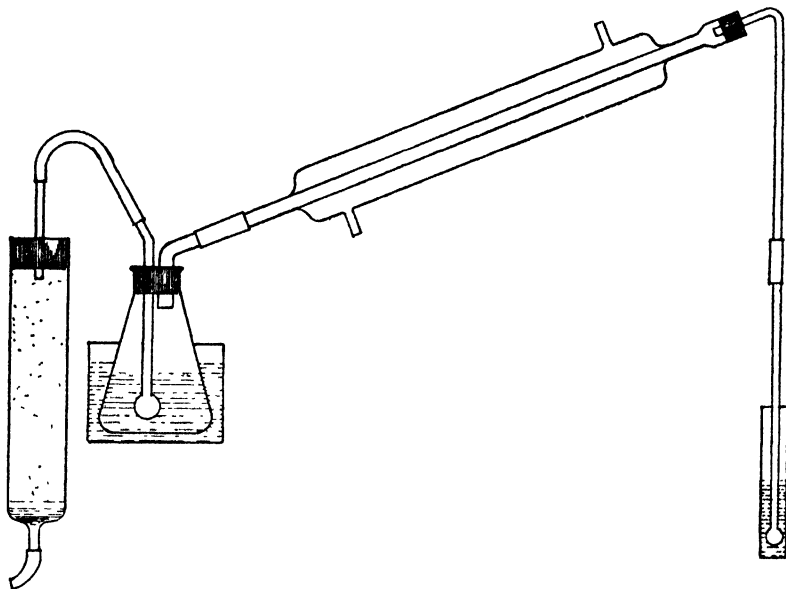
FIG. 1.—APPARATUS FOR DETERMINATION OF HCN BY AERATION METHOD.

ing the isolation by aeration and determination by the basic titration method, resulted. The apparatus set-up is shown in Figure 1.

DETERMINATION OF HYDROCYANIC ACID IN FOODS

Fit the aeration flask (300 cc. Erlenmeyer) with a two-holed No. 6 rubber stopper. Through one of the perforations pass a spray tube having a bulb at the lower end containing a number of small holes, and extend it nearly to the bottom of the flask. In the other perforation place a 9 mm. (internal diameter) glass tube, terminating just below the stopper and extending about an inch above it. Connect this latter tube, by means of a piece of 9 mm. rubber tubing, to a Liebig condenser inclined at about 45°. Connect the spray tube to the soda lime tower and this in turn to the compressed air outlet by means of rubber tubing. Fit the upper end of the condenser with a one-holed rubber stopper carrying a glass tube bent to extend vertically downward. Connect the latter to a spray tube, which is immersed nearly

to the bottom of the absorbing liquid contained in a 15–20 mm. by 6 inch glass receiving tube. (The receiving tube should have a flat bottom if the greatest sensitivity is desired. For ordinary determination a 6 X ⅝ inch (15 mm.) test tube may be used.)

Place 11 cc. of water and 2.0 cc. of 10% NaOH in the tube to absorb the HCN before placing the sample in the aeration flask. (For very small amounts use only 1 cc. of 10% NaOH and 8 cc. of water in a 15 mm. flat-bottomed tube.) Before weighing out the sample adjust the compressed air outlet so that there is a flow of 800–1000 cc. per minute, tested by collecting the air over water in a liter flask and using a stop-watch.

Thoroughly grind the sample in a mortar or food chopper and weigh 25 grams directly into the flask, or on a small filter paper in the case of dried fruits. Add 180 cc. of H_2SO_4 (1 +50), stopper the flask with a solid rubber stopper, and shake a few minutes to partially break up the sample. Rinse off the stopper with a little water and connect the flask with the apparatus by means of the stopper carrying the spray and distillation tubes, connect to the condenser, and raise the flask into place. Place a small bath so that the lower part of the aeration flask is immersed to the depth of 1–1.5 inches in a 35% glycerol solution. Immediately connect the air flow, heat the bath rapidly to about 104° C., and then lower the flame so that the temperature remains at 105°–110° C. After the temperature of the bath reaches 100° C., continue the flow of air for 15 minutes. then remove the flame and disconnect the air from the distillation flask. Disconnect the spray tube but allow it to remain in the absorption solution to be used as a stirrer. Add 2 cc. of NH_4OH (1 +2) and 1 cc. of 5% KI and titrate with 0.02 N AgNO$_3$ to the appearance of a turbidity, using a black background. 1 cc. of the 0.02 N AgNO$_3$ is equal to 1.08 mg. of HCN.

RECOVERIES BY THE METHOD

To test the recovery of the procedure, known quantities of potassium cyanide were added to food samples. Although this practice may not be entirely in accord with food samples containing hydrocyanic acid obtained on the market, there appeared to be no other practical way of adding a definitely known quantity of the hydrocyanic acid.

The quantities of potassium cyanide or hydrocyanic acid taken and the recoveries on samples of raisins are given in Table 1.

The results given in the table indicate that the method gives excellent recoveries when the directions are followed closely. Previous experiments

TABLE 1.—*Recovery of HCN from raisins*

SAMPLE	KCN ADDED	HCN EQUIVALENT	HCN FOUND	RECOVERY
	mg.	*mg.*	*mg*	*per cent*
1	10.00	4.15	4.18	100.7
2	1.00	0.415	0.438	105.6
3	40.00	16.60	16.3	97.3
4	40.00	16.60	16.6	100.0
5	40.00	16.60	16.65	100.3
6	20.00	8.30	8.15	98.2
7	4.00	1.66	1.66	100.0
8	20.00	8.30	8.28	99.7
9	40.00	16.60	16.35	98.5

on samples of raisins showed that a change in the order of adding the potassium cyanide and the acid made a difference in the recovery obtained. If water, then potassium cyanide, then acid were added, recoveries of only 75-85 per cent were obtained. If the acid were added before the potassium cyanide, the recoveries were the same as those given in the table. This difference is attributed to the cyanohydrin addition of some of the potassium cyanide to the aldehyde group of the dextrose in the raisins. This reaction proceeds much more rapidly at a high *p*H than at a low *p*H. The potassium cyanide or hydrocyanic acid thus changed is not recovered in the determination. However, in the proposed procedure sufficient acid is present to prevent the cyanohydrin reaction when the aqueous solution is added, and all hydrocyanic acid present should be recovered.

LIMIT OF ACCURACY OF THE BASIC TITRATION DETERMINATION

A minimum of about 8 cc. of absorbing solution containing 1 cc. of 10 per cent sodium hydroxide appears to be necessary to effect complete retention of all the hydrocyanic acid with the flow of air used in the method. A tube of about 10/16 inch in diameter, with a flat bottom, appears to be the best suited to the purpose in the Associate Referee's opinion. Using a tube of this kind containing 8 cc. of the absorption solution to which was added 2 cc. of ammonium hydroxide (1+2) and 0.5 cc. of 5 per cent potassium iodide he was able to distinguish the turbidity indicating the end point against a dark background with 0.02–0.03 cc. of 0.01 N silver nitrate, which quantity represents 0.011 mg. of hydrocyanic acid or is equivalent to 0.5 p.p.m. on a 25 gram sample. This should be satisfactory for most purposes.

NEW METHODS

Phenolphthalin test.—Among the more promising methods or tests is the phenolphthalin test.[2,3] While apparently not used to any extent in this country, this method has found considerable use in Great Britin and is claimed by Moffitt and Williams[2] to be the most sensitive test for hydrocyanic acid. The method is based on the oxidation of the colorless phenolphthalin to the indicator phenolphthalein by hydrocyanic acid, which gives the characteristic pink color in alkaline solution. The reagent is prepared according to Childs and Ball[3] as follows:

Ten cc. of a 1% solution of phenolphthalin containing 0.2% NaOH is diluted to 50 cc. with a 2.5% solution of glycerol. To this solution is added 50 cc. of a 0.3% solution of Cu acetate, and the whole is well mixed, and filtered if cloudy, and kept in a stoppered glass bottle. The reagent must be freshly prepared. One cc. of the reagent is added to the solution to be tested and this made basic with NaOH.

[2] *Analyst*, 62, 101-6 (1937).
[3] *Ibid.*, 60, 294 (1935).

Phenolphthalin qualitative test.—This reagent is well adapted to the qualitative detection of hydrocyanic acid as well as to its quantitative determination. In the opinion of the Associate Referee the reagent is much superior to the gum guaiac qualitative test for hydrocyanic acid. The following simple qualitative test may be made on such foods as dried fruits in a manner similar to the guaiac test.

Place 30–50 grams of sample in a 200 cc. Erlenmeyer flask and close with a rubber stopper for 20–30 minutes. Then moisten a strip of filter paper with a dilute NaOH solution (5%), raise the stopper, and quickly insert the strip and suspend it by the end that is held between the stopper and the flask. Remove the strip after 45 seconds, and place a drop or two of the reagent on it. Immediately there is produced the pink of phenolphthalein in alkali if cyanide is present. If the paper remains white, no cyanide is present. The test is not specific for cyanide, however, as free halogens will also give the test and possibly other volatile oxidizing agents.

Phenolphthalin quantitative determination of cyanide.—The phenolphthalin reagent can be added directly to the solution in which the hydrocyanic acid has been absorbed in the aeration method after making to definite volume. The color developed is compared with standards containing the same amount of alkali and reagent in an equal volume of solution. As stated above, the test is not specific for cyanide but ferricyanides and aqueous solutions of the halogens give a similar coloration. Sulfides interfere and if present should be removed by addition of lead nitrate or acetate before distillation or aeration. Ferrocyanide, chromates, nitric acid, ferric chloride, and halogen salts give no reaction with the reagent.[3]

Photometric method.—Because of the nature of the color produced, it was thought that the phenolphthalin method would lend itself readily to photometric determination, but several difficulties were encountered in attempting to do this. The pink color in aqueous solution was found to fade rapidly so as to make a definite reading almost impossible. In alcoholic solution the color was found to be much less intense than in aqueous solution and gradually increased in intensity upon standing.

Experimentation showed that in 30 per cent alcohol the color was nearly as intense as in water, and the color remained quite constant for an hour or more. Standards developed in 50 cc. of 30 per cent alcohol containing progressive amounts of hydrocyanic acid showed that the color produced was not a linear function of the hydrocyanic acid present. The curve produced is given in Figure 2 and appears to be parabolic. The readings were made in a Clifford and Brice type photometer, *This Journal*, **19**, 132 (1936), with a one-inch cell. The photometric method offers considerable promise, in the opinion of the Associate Referee, and may afford a way of reaching the near gamma range by decreasing the volume in which the color is developed and increasing the length of the cell. A No. 56 light filter should be used.

Thiocyanate method.—An effort was made to devise a thiocyanate

method since this would be delicate and sensitive and practically specific for cyanide. Criticisms of the ferric thiocyanate color in the past have been its rapid fading and variation in different acid strengths. A recent method for iron[4] based on the thiocyanate reaction appeared to offer a solution to the instability of the color. Instead of developing the color in aqueous solution it was done in 2 methoxy ethanol. In the latter solution the color was claimed to be more intense and also permanent.
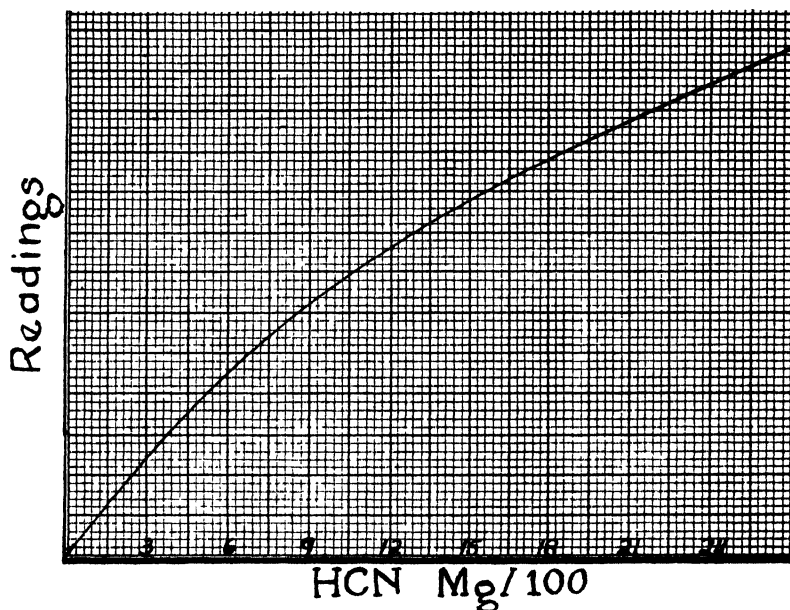
FIG. 2.

An attempt was made to reverse the reagents and use a similar determination for cyanide. To convert the hydrocyanic acid to thiocyanate it is necessary, of course, to evaporate the solution to dryness with ammonium polysulfide or sodium sulfide. Some determinations in which the residue was taken up in a few cc. of 1 to 1 methyl cellosolve and water gave good results in some cases, but the presence of dissolved sulfur offers some objection and causes a fading of the color. The present problem is to find a way of removing all sulfur without too much manipulation. The method appears to give promise for micro quantities if the sulfur can be removed without too much difficulty.

RECOMMENDATIONS[5]

It is recommended—

(1) That the recoveries of hydrocyanic acid by aeration and basic titration given in this report be checked by other analysts.

[4] Ind. Eng. Chem. Anal. Ed., 9, 453 (1937).
[5] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 62 (1939).

(2) That the phenolphthalin method given in this report be further studied.

(3) That the work on a thiocyanate method be continued.

REPORT ON FRUITS AND FRUIT PRODUCTS

By B. G. HARTMANN (U. S. Food and Drug Administration,
Washington, D. C.), *General Referee*

This year's schedule on the subject of Fruits and Fruit Products lists five recommendations. Time did not permit of work on all the assignments, but progress has been reported on most of them. Much of the work is preliminary in character.

SOLUBLE SOLIDS AND EFFECTS OF ACIDS ON SUGARS ON DRYING

This assignment has been carried by the Association for several years without report.

The determination of soluble solids in fruit and fruit products has been the subject of extensive investigations by H. J. Wichmann, Kathryn Breen, P. L. Gowan, T. Macara, L. H. McRoberts, and P. A. Clifford. McRoberts, *This Journal*, 16, 374 (1932), briefly summarizes the work of these investigators, and the report by Clifford appears in 17, 215 (1934).

From supporting evidence obtained in these investigations the general conclusion is reached that of the methods studied the refractometric procedure is best suited for the determination of soluble solids in fruits and fruit products.

T. Macara[1] pointed out that in the examination of solutions containing invert sugar, glucose solids, and citric acid, corrections are necessary when the Schönrock tables for sucrose are used. McRoberts corroborated Macara's findings, and in consequence of his work he was appointed by the Association to study this phase of the project. In 1934 McRoberts reported sufficient experimental work to show conclusively that for the determination of the soluble solids of fruit products by means of the refractometric index, the Schönrock table for sucrose is not applicable without correction in the case of products high in invert sugar, such as jellies. McRoberts was not able to follow up the work, and for the past few years no report has been received on the subject.

The Beverage Section of the Food and Drug Administration, Washington, D. C., recommends the use of the refractometric procedure for the determination of soluble solids in preserve and jelly work. The method, it is believed, is at least as accurate as methods based on evaporation of an aliquot of the sample solution and drying the residue in vacuo at 70° C. It has the further advantage of requiring less time than do drying methods.

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[1] *Analyst*, 56, 391 (1931).

Apparently the refractometric method is suitable for regulatory needs, despite the fact that in the evaluation of solids, the Schönrock sucrose tables are used. In view of the fact that the refractometric method has been used in official work for several years with entire satisfaction, there seems to be no immediate necessity of pursuing the rather involved study necessary to correct the hitherto serviceable Schönrock table, and it is recommended that the work on soluble solids be dropped for the present.

PROPOSED CHANGES IN THE ANALYSES OF PRESERVES AND JAMS

In the paper, entitled "Chemical Analysis of Preserves and Jams," by J. W. Sale, *This Journal*, 21, 503 (1938), modifications of the methods used for analyzing preserves and jams are discussed. They are the result of several years of experience in the examination of these products and are offered for the purpose of correcting the apparent uncertainties existing in the mind of the analyst regarding the provisions of some of the official procedures. As these modifications do not affect the principles of the official procedures, but apply only to changes and additions in their text, it seems unnecessary to submit them to collaborative study. Accordingly, they are recommended for adoption as official (first action). The changes were published in *This Journal*, 22, 78 (1939).

Much thought has been given to the improvement of the official methods for the determination of the insoluble solids in products containing pits and large seeds, such as damson plums and grapes. It is evident that the 25 gram sample (official method) of a damson plum preserve, with its large pits, is not conducive to an accurate determination. In 1936 Samuel L. Alfend of the St. Louis Station, *This Journal*, 20 (1937) proposed the following procedure for fruits of this type:

Pulp the entire material as thoroughly as possible in a mortar without grinding the seeds or pits, or attempting to break up the skins. Weigh out 200 grams of actual fruit, or, in the case of preserves, jams, etc., weigh out 300 grams of sample, boil with water for the usual time, filter at once through a weighed cotton pad, wash until the wash water is free of solids, and make to 2 liters. After obtaining the weight of the dried material, pick out seeds or pits and soak them thoroughly in water to remove adhering insoluble matter. Rinse, dry, and subtract their weight from the entire weight of insoluble solids.

Obviously this procedure should give a more accurate result than that which is obtained by the official 25 gram sample. No collaborative work has been done on this important subject, and it is therefore suggested that in the coming year the above method be made the subject of collaborative study.

INACTIVE MALIC, ISOCITRIC, AND LACTIC ACIDS

No collaborative study on the determination of inactive malic acid and lactic acid was made.

The hope expressed in the Referee's last report regarding a possible method for isocitric acid did not materialize. It developed that before any progress could be made in the devising of a method, it would be necessary to find some means of removing tannin and coloring matter from the sample. As it was believed that charcoal would answer the purpose, an investigation was made with "Nuchar W," one of the charcoals used in analytical procedures. The results are shown in Table 1.

TABLE 1.—*Adsorption of organic acids on charcoal*
Nuchar (W), 0.200 gram. Volume of Solution, 110 cc. at 20°. Time, 2 Minutes

ACID	ACID ADSORBED (PER CENT)				
	SUCCINIC	CITRIC	MALIC (LEVO)	TARTARIC	ACETIC
grams in 110 cc. soln					
.0125	57.6	52.0	45.6	40 8	39.2
.0376	34.3	33 5	26.9	23 1	15 7
0626	24.8	23.0	18 7	15.8	11.5
.0875	19.2	17 9	15 0	13 7	9.4
.1125	16.4	14.9	12.9	12.3	7.6
.1375	14.1	12 5	11 1	9.5	6.6
.1625	12.6	11 0	9 8	8 4	6.2
.1875	11.0	9.9	8.8	7.6	5 5
.2125	10.1	9 1	8 1	6 8	5 0
.2377	9.2	8.0	7.4	6 3	4.6

The data (Table 1) require no comment. They show conclusively that charcoal is not suitable for the quantitative determination of acids, particularly when the acid concentration is low.

TOTAL ACIDS (FREE AND COMBINED)

In the course of the investigation of methods for the determination of malic, inactive malic acid, and isocitric acids, it became necessary to determine accurately the sum of these acids (free and combined).

After considerable experimentation it was found that the lead salts of the polybasic acids, ordinarily found in fruits after removal of lead with hydrogen sulfide, yielded solutions that could be accurately titrated for their total acid content.

Fifty cc. of the solution containing the quantity of acid indicated (Table 2) was pipetted into a centrifuge bottle. Lead acetate solution and about 200 cc. of 95 per cent alcohol were added, and the mixture was shaken vigorously for about 2 minutes and centrifuged. The supernatant liquor was decanted, and the lead salts were washed with 80 per cent alcohol decomposed with hydrogen sulfide, transferred to a 110 cc. volumetric flask, made to mark with water, and filtered; 100 cc. of the clear filtrate was transferred to a 400 cc. beaker, evaporated to about 50 cc. and titrated with 0.1 N alkali.

MALIC ACID

A question often arises as to the necessity in the official method for determination of malic acid in fruit products, *Methods of Analysis A.O.A.C.*, 1935, 328, for subjecting the sample solution to the rather tedious "tribasic lead acetate" step. The answer to the question depends entirely on the nature of the acids contained in the material under examination.

Since the final determination of the acid involves a polarimetric reading, it is necessary to remove all interfering, optically-active compounds, such as sugars, tartaric and isocitric acids, pectin, and tannins, before the polarization is undertaken. The removal of sugars, pectin, and tartaric acid is easily accomplished, but iso-citric acid, tannin, and coloring matter require the treatment with tribasic lead acetate. Tannin and coloring

TABLE 2.—*Recovery of acids from lead precipitate*

ACID	ACID TAKEN	ACID RETURNED		ACID RETURNED
	mg.	*mg.*		*per cent*
Citric	99.3	99.1 98.9	99.0	99.7
Tartaric	99.4	99.0 99.2	99.1	99.7
Malic	98.2	96.8 97.2	97.0	98.8
Succinic	99.4	98.6 98.9	98.8	99.4
Citric and Tartaric	49.7 49.7 (99.4)	99.6 99.3	99.5	100.1
Potassium Acid Tartrate	124.5	124.0 124.0	124.0	99.6

matter are present in all fruit products; isocitric acid has been found only in the blackberry. Obviously, tannin and coloring matter are the chief offenders in the determination of malic acid. Tannin, besides being optically active, also exhibits a distressing tendency to produce brownish solutions when treated with uranium acetate, thus still further vitiating polarimetric readings.

There is at present no method available for the removal of tannin. Treatment with charcoal is not suitable for the purpose, as was shown elsewhere in this report. Experiments with zinc salts for the precipitation of tannin were not successful.

Until a procedure for removing tannin has been devised it will be necessary to subject the sample to the tribasic lead acetate treatment. In the analysis of samples known to be free of isocitric acid and low in tannin it should be permissible to omit the step in cases where an accurate determination is not desired. Recent work has shown that it may be possible to shorten the malic acid method.

It has been found that the zinc salts of citric and isocitric acids are precipitable in 75 per cent alcohol, while those of malic acid are readily soluble in this concentration of alcohol. In case the separation is found to be quantitative the further advantage of an increase in the quantity of sample, which is now restricted through the empiricism of the present method, will have been gained. This is important in cases where the total acid is relatively high but the content of malic acid is low, as in currants, grapefruit, pineapples, and raspberries.

CITRIC ACID BY THE PENTABROMACETONE METHOD

The official method for the determination of citric acid in foods by pentabromacetone has been criticized in the literature, particularly that part of it which deals with the oxidation of the brominated solution with permanganate.

In a recent article R. S. Paul[1] has the following to say regarding the determination of the acid:

The pentabromacetone method is now coming to be generally recognized as the most satisfactory available. None of the descriptions of the method given in the literature was entirely satisfactory, the best at the time being that of the A.O.A.C., which allows for the slight solubility of pentabromacetone in water. It is further stated that the purity of the pentabromacetone depends on the care with which the oxidation is carried out. Rapid oxidation tends to the formation of products which are largely of such a degree of volatility as to interfere with the drying to a constant weight in a vacuum desiccator.

In an investigation by Lampitt and Rooke,[2] the following conclusions are reached regarding the oxidation of citric acid and the collection of the pentabromacetone:

1. There is no important difference between the results whether 50 ml. or 100 ml. of original citric acid solution are taken for analysis, but owing to the solubility of pentabromacetone it is advisable to keep the volume of the reaction solution as low as possible.

2. The temperature of the oxidation is immaterial (below 50° C.) but at lower temperature a longer reaction time is necessary.

3. Too rapid addition of permanganate is harmful, but excess does not matter; more ferrous sulfate solution, however, is then necessary.

4. It is advisable to cool the reaction mixture in the ice-chest overnight before filtration.

5. The volume of wash water should be kept as low as possible—not more than 25 ml.

Apparently the official method, with the exception of the manner of oxidizing the brominated citric acid solution, fulfils the requirements cited in the above reference.

Data obtained by rapid and slow oxidation of solutions containing known quantities of pure citric acid are presented in Table 3.

[1] *Analyst,* **60,** 635 (1935).
[2] *Ibid.,* 61, 654 (1936).

The results recorded under "Rapid Oxidation " (Table 3) were obtained by following the requirements for the oxidation of citric acid and the collection of the pentabromacetone laid down in the official method. For those under "Slow Oxidation" the same procedure was followed except that the permanganate was added slowly instead of all at once. Following bromination the permanganate was added from a buret in 2 cc. portions, and the mixture was shaken a few seconds after each addition. After the introduction of the 25 cc. permanganate solution, which required about 1.5 minutes, the mixture was shaken about 1 minute and allowed to stand for 3 minutes. The ferrous sulfate solution was then added and the pentabromacetone determined as directed in the official method.

TABLE 3.—*Rapid and slow oxidation of citric acid*

| CITRIC ACID IN SOLUTION | CITRIC ACID DETERMINED* | |
	RAPID OXIDATION .424 P (MG.)	SLOW OXIDATION .424 P (MG.)
mg. 1.53	No precipitate	Slight precipitate
3.89	1.31 1.53 Av. 1.42	1.82 1.82 Av. 1.82
7.78	4.79 4.75 Av. 4.77	5.43 5.55 Av. 5.49
38.29	32.61 32.99 Av. 32.80	34.48 34.68 Av. 34.58
114.87	103.62 103.96 Av. 103.79	108.33 108.54 Av. 108.44

* The volume of the reaction mixture was about 150 cc. in all cases; 25 cc. each of $KMnO_4$ and $FeSO_4$ solution was used.

The results show that slow oxidation yields materially more pentabromacetone than does rapid oxidation. This circumstance would indicate that the loss of pentabromacetone in the official method is not due entirely to solubility but in part either to incomplete oxidation of citric acid or to the formation of volatile bromine compounds. It was noticed that the pentabromacetone obtained in slow oxidation was more crystalline than that produced in rapid oxidation, a circumstance that makes for better filtration and drying.

There can be no question that slow oxidation is preferable to rapid oxidation. However, more work is needed before a revised procedure for this all important step can be written into the official method; apparently the solubility factor is materially smaller with slow oxidation.

It is recommended that the slow versus rapid oxidation be investigated as to preference in the conversion of citric acid into pentabromacetone.

POLARISCOPIC METHODS FOR JAMS, JELLIES, AND PRESERVES

In 1935 the Referee on Sugar and Sugar Products pointed out that certain constituents of food products, notably pectins, interfere with the polarimetric determination of sugars and recommended a study of the effect of clarifying agents for the purpose of their elimination.

The necessity for such a study is evident when it is considered that food products very frequently contain large quantities of interfering optically active compounds other than pectins, which if not removed will cause serious errors in sugar determinations.

Fortunately the lead reagents generally used for clarifying sugar solutions precipitate the majority of these interfering substances, but owing to the solubility of the lead salts in aqueous solution their removal is far from quantitative. It would seem, therefore, that in order to suppress solubility other vehicles than water should be tried. It has been found that the lead salts of pectin and of the acids are almost completely removed in 70 per cent alcohol.

Although it is realized that the possible occlusion of sugars in such a system may vitiate the determination, it is believed that the idea is deserving of a trial.

It is recommended that this study be continued.

ELECTROMETRIC TITRATION OF ACIDS

In continuation of last year's work, the associate referee reports progress in the development of fundamental data for the interpretation of titration curves obtained on mixtures of fruit acids.

It is recommended that this work be continued next year.

P_2O_5 IN JAMS, JELLIES, AND OTHER FRUIT PRODUCTS

No report was received from the associate referee on this assignment. It is recommended that this study be continued next year.

RECOMMENDATIONS[1]

It is recommended—

(1) That study of soluble solids be discontinued for the present.

(2) That study of electrometric titration be continued.

(3) That the changes in the official methods for the analysis of preserves and jams indicated in this report be considered for adoption.

(4) That study of methods for inactive, malic, isocitric, and lactic acid be continued.

(5) That the effect of slow oxidation on the yield of pentabromacetone in the determination of citric acid be studied.

[1] For report of Subcommittee D and action by the Association, see *This Journal* **22**, 66 (1939).

(6) That study of polarimetric methods for jams, jellies, and preserves be continued.

(7) That the study of P_2O_5 in jams, jellies, and other fruit products be continued.

No report on soluble solids and effects of acids on sugar on drying was given by the associate referee.

REPORT ON ELECTROMETRIC TITRATION OF ACIDITY

By ROBERT U. BONNAR (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

During the past two years the Associate Referee has considered the problem of measuring the components of a mixture of weak acids from the titration curve of the mixture. As the introductory step, the mathematical basis of such measurements has been developed from the buffer properties of weak acids.

At constant temperature and constant ionic strength, the pH of a partially neutralized solution of a weak monobasic acid of concentration C and dissociation constant K is given by

$$1. \qquad pH = pK + \log \frac{X}{C - X}, [1]$$

where $pK = \log 1/K$ and X is the concentration of the salt of the weak acid arising from neutralization. If several buffer solutions be formed of one initial acid concentration, pH of the j-th buffer solution is

$$2. \qquad pH_j = pK + \log \frac{X_j}{C - X_j},$$

X_j being the concentration of neutralized acid in the j-th solution. Now, if i monobasic acids be present, the equation becomes

$$3. \qquad pH_j = pK_i + \log \frac{X_{ij}}{C_i - X_{ij}},$$

X_{ij} being the concentration of the i-th acid taken up by neutralizer in the j-th buffer solution. This equation may now be solved for X_{ij}. Letting $W_{ij} = pH_j - pK_i$

$$\frac{X_{ij}}{C_i - X_{ij}} = 10^{W_{ij}}$$

$$X_{ij} = 10^{W_{ij}}C_i - 10^{W_{ij}}X_{ij},$$

$$4. \qquad = \frac{10^{W_{ij}}}{1 + 10^{W_{ij}}} C_i.$$

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[1] W. M. Clark, The Determination of Hydrogen Ions, 3rd Ed., 1928, Chapter I, p. 22, Eq. 23.

5.   Let $r_{ij} = \dfrac{10^{W_{ij}}}{1 + 10^{W_{ij}}}$,   then

6. $X_j = r_{ij}C_i.^2$

Here X_j is the total concentration of neutralized acid, corresponding to the number of equivalents of alkali added in titration, at $p\mathrm{H}_j$. The titration curve is thus represented by the formation of successive buffer solutions of i acids, each having its own dissociation constant and present in its own concentration. From j points along the titration curve, a set of j simultaneous equations in i unknowns can be constructed, which will allow the determination of each of the i acids, with an estimate of error based on $j-i$ degrees of freedom.

The chemical measurements available are X, the number of equivalents of alkali added, and the $p\mathrm{H}$ of the solution. In order to obtain r for an unknown sample, the dissociation constants of the acids present must be known. Then $W_{ij} = p\mathrm{H}_j - p\mathrm{K}_i$ must be calculated for each particular acid. Table 1 has been constructed to give r for positive values of W from the relation

5. $r = \dfrac{10^W}{1 + 10^W}$.

This table is applicable to any weak acid. Since r is tabulated only for the positive values[3] of W, and since the table is symmetrical about $W = 0$, $r = \frac{1}{2}$,

7. $r(-W) = 1 - r(\,|\,W\,|\,)$.

Experimentally, the problem is to determine W_{ij}. If the titration curve of known amounts of a pure acid be measured over the concentration range of interest, $p\mathrm{K}_i$ can be calculated, since the $p\mathrm{H}$ is known for each point of the curve.

This solution for $p\mathrm{K}_i$ is here called the "backward solution," to distinguish it from the solution for C_i, known as the "forward solution," which is the ultimate aim of the investigation. For monobasic acids, the backward solution is simple,

                    $X_j = r_jC$ and

8. $\dfrac{X_j}{C} = r_j$.

W_{ij} can be found by inverse interpolation in Table 1, and $p\mathrm{K}_i = p\mathrm{H}_j - W_{ij}$. Thus an average value of $p\mathrm{K}_i$ and a standard deviation with $j-1$ degrees of freedom can be directly calculated from the j measurements.

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[2] In this equation the tensor notation indicates summation according to the repeated subscripts. Thus, if $i = 1, 2\ 3, 4, 5$,
$$X_j = r_{1j}C_1 + r_{2j}C_2 + \cdots + r_{5j}C_5$$
[3] This artifice reduces by half the tabulation necessary. r for negative values of W is the difference between unity and r for the tabular absolute value of W.

$$\text{TABLE 1.} \quad r = \frac{10^W}{1+10^W}$$

w	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
3.0	.99900	.99902	.99905	.99907	.99909	.99911	.99913	.99915	.99917	.99919
2.9	.99874	.99877	.99880	.99883	.99885	.99888	.99890	.99893	.99895	.99898
.8	.99842	.99845	.99849	.99852	.99856	.99859	.99862	.99685	.99868	.99871
.7	.99801	.99805	.99810	.99814	.99818	.99822	.99827	.99830	.99834	.99838
.6	.99750	.99755	.99761	.99766	.99771	.99777	.99782	.99787	.99792	.99796
.5	.99685	.99692	.99699	.99706	.99712	.99719	.99725	.99732	.99738	.99744
.4	.99603	.99612	.99621	.99630	.99638	.99646	.99654	.99662	.99670	.99677
.3	.99501	.99513	.99524	.99534	.99545	.99555	.99565	.99575	.99585	.99594
.2	.99373	.99387	.99401	.99415	.99428	.99441	.99453	.99466	.99478	.99490
.1	.99212	99230	.99247	.99264	.99281	.99297	.99313	.99328	.99344	.99359
2.0	.99010	.99032	.99054	.99075	.99096	.99117	.99137	.99156	.99175	.99194
1.9	.98757	.98785	.98812	.98839	.98865	.98890	.98915	.98940	.98964	.98987
.8	.98440	.98475	.98509	.98542	.98575	.98607	.98638	.98669	.98699	.98728
.7	.98044	.98087	.98130	.98172	.98213	.98253	.98292	.98330	.98368	.98404
.6	.97550	.97604	.97657	.97709	.97760	.97810	.97859	.97907	.97953	.97999
.5	.96935	.97002	.97069	.97133	.97197	.97259	.97320	.97379	.97437	.97494
.4	.96171	.96255	.96337	.96418	.96497	.96573	.96649	.96723	.96795	.96865
.3	.95227	.95331	.95432	.95532	.95629	.95724	.95817	.95909	.95998	.96086
.2	.94065	.94192	.94317	.94439	.94559	.94676	.94791	.94903	.95014	.95122
.1	.92641	.92796	.92949	.93099	.93245	.93388	93529	.93667	.93803	.93935
1.0	.90909	.91098	.91282	.91464	.91642	.91817	.91988	.92156	.92321	.92482
0.9	.88818	.89045	.89268	.89486	.89701	.89912	.90119	.90322	.90521	.90717
.8	.86319	.86589	.86854	.87115	.87371	.87623	.87870	.88114	.88353	.88588
.7	.83366	.83683	.83995	.84302	.84604	.84902	.85195	.85483	.85766	.86045
.6	.79924	.80291	.80653	.81010	.81361	.81708	.82050	.82386	.82718	.83045
.5	.75975	.76392	.76805	.77213	.77616	.78013	.78405	.78792	.79175	.79552
.4	.71525	.71992	.72454	.72911	.73364	.73811	.74253	.74691	.75124	.75552
.3	.66614	.67124	.67630	.68133	.68629	.69123	.69613	.70097	.70578	.71054
.2	.61314	.61858	.62400	.62938	.63474	.64007	.64535	.65061	.65583	.66101
.1	.55731	.56298	.56863	.57429	.57990	.58549	.59107	.59663	.60216	.60767
0.0	.50000	.50576	.51150	.51726	.52301	.52875	.53449	.54021	.54593	.55161

For dibasic and polybasic acids, the titration is complicated by the different values of pK_1 and pK_2 for the different acids. Here, one form of the backward solution has been derived as follows—

6. $\qquad X_j = r_{ij}C_i.$ Let $i = 1$ and 2

9a. $\qquad X_j = r_{1j}C_1 + r_{2j}C_2$

for the same dibasic acid, $C_1 = C_2 = \frac{1}{2}C$, so

b. $\qquad X_j = \frac{1}{2}C(r_{1j} + r_{2j})$

c. $$\frac{2X_j}{C} = r_{1j} + r_{2j}.$$

Substituting the equivalents for r_{1j} and r_{2j} from Equation 5,

d. $$\frac{2X_j}{C} = \frac{10^{W_{1j}}}{1 + 10^{W_{1j}}} + \frac{10^{W_{2j}}}{1 + 10^{W_{2j}}}.$$

Let $10^{W_{1j}} = \dfrac{1}{S}$, $10^{W_{2j}} = \dfrac{1}{N}$, $\dfrac{2X_j}{C} = R$.

Then substituting and clearing,

10. $$R = \frac{1}{S+1} + \frac{1}{N+1}. \qquad = \frac{N + S + 2}{NS + S + N + 1}.$$

The next step is to proceed to curve-fitting.[4]

In the process of curve-fitting, there is a function F_0 such that, for this case,

11a. $F_0 = N + S + 2 - RNS - RS - RN - R.$

b. $\qquad = (1 - R)N + (1 - R)S + (2 - R) - RNS$

Now, for N, substitute its equivalent $10^{-W_{2j}} = 10^{(p\mathrm{K}_2 - p\mathrm{H}_j)}$

$$= \frac{10^{p\mathrm{K}_2}}{10^{p\mathrm{H}_j}}, \quad \text{and for } S, \quad \frac{10^{p\mathrm{K}_1}}{10^{p\mathrm{H}_j}}$$

12. $$F_0 = \frac{1 - R}{10^{p\mathrm{H}_j}} \cdot 10^{p\mathrm{K}_2} + \frac{1 - R}{10^{p\mathrm{H}_j}} \cdot 10^{p\mathrm{K}_1} + (2 - R) - \frac{R \cdot 10^{p\mathrm{K}_1} \cdot 10^{p\mathrm{K}_2}}{10^{2p\mathrm{H}_j}}$$

Let $\dfrac{1 - R}{10^{p\mathrm{H}}} = Y_1$, $\dfrac{R}{10^{2p\mathrm{H}}} = Y_2$, $2 - R = Y_3$

and $10^{p\mathrm{K}_1} = A$, $10^{p\mathrm{K}_2} = B$. Then

13a. $F_0 = Y_1 B + Y_1 A + Y_3 - Y_2 A B$

b. $\qquad = (A + B)Y_1 - ABY_2 + Y_3.$

Observe now that the measurements $R = 2X_j/C$ and the pH are combined to form the variables, while the pK values are involved in the parameters to be determined. In this solution, the exact value of the Lagrangian function must be used, instead of an assumed unity. The partial derivatives for curve-fitting are:

14. $\quad F'_A = Y_1 - BY_2 \qquad F'_{Y_1} = A + B$

$\quad F'_B = Y_1 - AY_2 \qquad F'_{Y_2} = -AB$

$\qquad\qquad\qquad\qquad F'_{Y_3} = 1.$

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[4] The process of curve-fitting here used is that of W. E. Deming, *Some Notes on Least Squares*, U. S. D. A. Graduate School, 1938, 181 pp. Other published works by Deming on the subject are *Phil. Mag.* 11, 146–158 (1931); 17, 804–928 (1934); 19, 389–402 (1935).

The Lagrangian function then becomes

$$15. \qquad L = \frac{(A + B)^2}{Wt.Y_1} + \frac{(AB)^2}{Wt.Y_2} + \frac{1}{Wt.Y_3} \cdot$$

The prior estimates A_0 and B_0 of A and B can be taken from existing tables, from publications, or from approximations from the titration curve. The weights are computed as follows;[5]

$$16a. \qquad \frac{1}{Wt.Y_1} = \frac{1}{Wt.R}\left(\frac{dY_1}{dR}\right)^2 + \frac{1}{Wt.pH}\left(\frac{dY_1}{dpH}\right)^2$$

$$\text{since} \quad Y_1 = \frac{1 - R}{10^{pH}} \qquad \frac{dY_1}{dR} = -10^{-pH}$$

$$\frac{dY_1}{dpH} = (1 - R)10^{-pH} \log_e 10 = (- \log_e 10)Y_1$$

$$b. \qquad \frac{1}{Wt.Y_1} = \frac{1}{Wt.R} \cdot 10^{-2pH} + \frac{Y_1^2(\log_e 10)^2}{Wt.pH},$$

where $Wt.R = n/S^2_R$, the reciprocal of the variance of the mean of R due to experimental error,

$Wt.pH = n/S^2_{pH}$, S^2_{pH} being the variance of the errors of pH measurement.

In like manner

$$17a. \qquad \frac{1}{Wt.Y_2} = \frac{1}{Wt.R}\left(\frac{dY_2}{dR}\right)^2 + \frac{1}{Wt.pH}\left(\frac{dY_2}{dpH}\right)^2$$

$$b. \qquad \frac{1}{Wt.Y_2} = \frac{1}{Wt.R} \cdot 10^{-4pH} + \frac{1}{Wt.pH}(2 \log_e 10)^2 Y_2^2$$

$$18. \qquad \frac{1}{Wt.Y_3} = \frac{1}{Wt.R} \cdot$$

The applicability of the "backward solution" for dibasic acids was tested with malic acid and succinic acids. Series of eighteen buffer solutions of approximately 0.01 M concentration in the acid were made by titration with sodium hydroxide and dilution to a uniform 50 cc. volume. These buffers were measured with the glass electrode. Sealed electrodes and the Beckman model F hydrogen ion meter standardized with 0.0500 M potassium biphthalate at pH 4.00 at 25° C. were used. S^2_R was experimentally determined as 0.0119 for nine degrees of freedom. S^2_{pH} was assumed to be 0.0001 when $S = 0.01$ pH was used as the least count of the instrument. A_0, the prior estimate of A, was taken as $10^{4.18}$ for suc-

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[5] Deming, Least Squares, p. 30, Ex. 11.

cinic acid and $10^{3.48}$ for malic acid. B_0, the prior estimate of B, was taken as $10^{5.57}$ for succinic acid and $10^{5.11}$ for malic acid.[6] pK_1 was then found to be 4.20 for succinic acid and 3.48 for malic acid, with standard devia-

TABLE 2. $\left(\dfrac{dr}{dW}\right)^2 = 5.30190\ r^2(1-r)^2$ for values of r from Table 1

w	.00	.01	.02	.03	.04	.05	.06	.07	.08	.09
3.0	.00001	.00001	.00000	.00000	.00000	.00000	.00000	.00000	.00000	.00000
2.9	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001
.8	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001	.00001
.7	.00002	.00002	.00002	.00002	.00002	.00002	.00002	.00002	.00001	.00001
.6	.00003	.00003	.00003	.00003	.00003	.00003	.00003	.00002	.00002	.00002
.5	.00005	.00005	.00005	.00005	.00004	.00004	.00004	.00004	.00004	00003
.4	.00008	.00008	.00008	.00007	.00007	.00007	.00006	.00006	.00006	.00005
.3	.00013	.00012	.00012	.00011	00011	.00010	.00010	.00009	.00009	.00009
.2	.00021	.00020	.00019	.00018	.00017	.00016	.00016	.00015	.00014	.00014
.1	.00032	.00031	.00030	.00028	.00027	.00026	.00025	.00024	.00023	.00022
2.0	.00051	.00049	.00047	.00045	.00043	.00041	.00039	.00037	.00035	.00034
1.9	.00080	.00076	.00073	.00070	.00067	.00064	.00061	.00058	.00056	.00053
.8	.00125	00120	.00114	.00109	.00105	.00100	.00096	.00091	.00087	.00084
.7	.00195	.00187	.00179	.00171	.00163	.00156	.00149	.00143	.00137	.00131
.6	.00303	.00290	.00278	.00266	.00254	.00243	.00233	.00223	.00213	.00204
.5	.00468	.00448	.00429	.00411	.00394	.00377	.00361	.00345	.00331	.00316
.4	.00719	.00689	.00660	.00632	.00606	.00581	.00556	.00532	.00510	.00489
.3	.01095	.01050	.01008	.00966	.00926	.00888	.00851	.00816	.00783	.00750
.2	.01652	.01587	.01523	.01462	.01403	.01347	.01293	.01241	.01190	01142
.1	.02464	.02369	.02277	.02188	.02103	.02022	.01942	.01865	.01791	.01721
1.0	.03621	.03487	.03358	.03232	.03110	.02993	.02880	.02770	.02665	.02563
0.9	.05230	.05045	.04866	.04693	.04525	.04362	.04204	.04051	.03904	.03760
.8	.07394	.07150	.06912	.06680	.06455	.06236	.06023	.05816	.05614	.05419
.7	.10195	.09885	.09582	.09285	.08996	.08712	.08435	.08165	.07902	.07644
.6	.13650	.13277	.12909	.12548	.12193	.11844	.11501	.11165	.10835	.10511
.5	.17664	.17244	.16827	.16413	.16003	.15599	.15199	.14805	.14414	.14029
.4	.21993	.21556	.21119	.20683	.20246	.19811	.19378	.18946	.18516	.18089
.3	.26224	.25819	.25409	.24994	.24576	.24152	.23724	.23295	.22862	.22428
.2	.29830	.29514	.29186	.28848	.28499	.28140	.27773	.27396	.27012	.26621
.1	.32272	.32094	.31900	.31690	.31466	.31228	.30975	.30708	.30428	.30135
0.0	.33137	.33128	.33102	.33058	.32997	.32918	.32822	.32710	.32580	.32435

tions of 0.008 and 0.014 based on sixteen degrees of freedom, respectively; pK_2 was found to be 5.41 for succinic acid and 4.93 for malic acid with standard deviation (sixteen degrees of freedom) of 0.01 and 0.013, respectively. This shows that pK values can be found from glass electrode

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[6] Clark, W. M. Determination of Hydrogen Ions, p. 678.

measurements of a titration curve to within the least count of present-day instruments.

These values were then used with nine-point titration curves in two attempts to resolve mixtures of approximately equal parts of malic and succinic acids.

Reverting to Equation 6, for two dibasic acids this becomes:

19. $$X_j = r_{1j}C_1 + r_{2j}C_1 + r_{3j}C_2 + r_{4j}C_2.$$

The curve-fitting equation is:

20. $$F_0 = (r_1 + r_2)C_1 + (r_3 + r_4)C_2 - X.$$

The partial derivatives of F_0 are:

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a. $F'_{C_1} = r_1 + r_2$ e. $F'_X = -1$

b. $F'_{C_2} = r_3 + r_4$ f. $F'_{r_3} = C_2$

c. $F'_{r_1} = C_1$ g. $F'_{r_4} = C_2.$

d. $F'_{r_2} = C_1$

The Lagrangian function is:

22. $$L = \frac{C_1{}^2}{Wt.r_1} + \frac{C_1{}^2}{Wt.r_2} + \frac{C_2{}^2}{Wt.r_3} + \frac{C_2{}^2}{Wt.r_4} + \frac{1}{Wt.X}$$

where

$$\frac{1}{Wt.r} = \frac{1}{Wt.W}\left(\frac{dr}{dW}\right)^2$$

24a. since $$r = \frac{10^W}{1 + 10^W}, \qquad Ln\ r = Ln\ 10^W - Ln(1 + 10^W)$$

b. $$\frac{dr}{r} = \frac{2.303\ 10^W}{10^W}\ dW - \frac{2.303\ 10^W}{1 + 10^W}\ dW$$

c. $$= 2.303(1 - r)dW$$

d. $$\frac{dr}{dW} = (2.303)r(1 - r)$$

25. $$\frac{1}{Wt.r} = \frac{1}{Wt.W}\ (2.303r)^2(1 - r)^2.$$

Table 2 gives $(dr/dW)^2$ for the values of r given in Table 1. Since dr/dW is symmetrical with respect to $r = \frac{1}{2}$, the entry in Table 2 for r greater than .5 is also the entry for $1 - r$ greater than .5, and so, as tabulated, for the absolute value of W.

Recalling Equation 19, it is noted that, since dibasic acids are involved, C_1, the number of equivalents of the first hydrogen of malic acid, is equal to the number of moles of the acid, and likewise C_2 is both the number of equivalents of the first hydrogen and the number of moles of succinic acid. Therefore molal concentrations may be used. Table 3 shows the

TABLE 3

TRIAL	ACID	PRIOR ESTIMATE	PRESENT	FOUND
		moles/liter	*moles/liter*	*moles/liter*
I	Malic	.00505	.00481	.00473
	Succinic	.00505	.00497	.00508
	Total	.0101	.00978	.00981
II	Malic	.00606	.00577	.00555
	Succinic	.00404	.00398	.00419
	Total	.0101	.00975	.00974

prior estimates, the concentrations present, and the concentrations found in these two experiments. Since the full least squares solution, involving the Lagrangian function, was necessary, the statistical weights are given by

$1/Wt.X = 1.349066 \times 10^{-10}$ by conversion of $Wt.R$, to a concentration basis

$1/Wt.W = S^2_{pH} + S^2_{pK}/K$ where S^2_{pH} and S^2_{pK} are taken from the "backward solution" for $K = 16$ degrees of freedom,

$1/Wt.W = 1/53334$ for malic acid and

$1/Wt.W = 1/80000$ for succinic acid.

It is believed that these experiments, although under conditions too idealized to be regarded as constituting a method of analysis, confirm the usefulness of this approach to the determination of organic acids in mixtures. It is therefore recommended that the studies of the electrometric titration curve be continued.

For report on malic, isocitric, and lactic acids, see Report of the Referee on Fruits and Fruit Products.

No report on polariscopic methods for jams, jellies, and preserves was given by the associate referee.

No report on P_2O_5 in jams, jellies, and other fruit products was given by the associate referee.

REPORT ON CANNED FOODS

By V. B. BONNEY (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

During the past packing season the Canned Food Section of Food Division of the Food and Drug Administration collaborated with the National Canners Association laboratory in San Francisco, in experimental packs of canned Royal Anne cherries, whole unpeeled apricots, clingstone peaches—both halves and slices—and Bartlett pears in halves. In these studies a large number of packs of 36 cans each were prepared, and the exact put-in weight of fruit in each can was recorded. The packs covered variations in packing medium (water, or sugar sirups of various strengths), degrees of ripeness of fruit, fruit from different growing conditions, different methods of cooking, and the different sizes of cans commonly used.

The plans for these studies include an arrangement whereby 3 cans from each pack are examined the 60th, 90th, and 180th days after packing in the National Canners Association laboratory in San Francisco and 3 other cans are examined the same days in the Canned Food Section laboratory in Washington. In order to insure uniform procedure the following method is being used in each of the laboratories for the determination of drained weight, one of the most important factors in the studies:

Pour the entire contents of the container on a round sieve with a No. 8 standard screen (diameter of wire 0.84 mm. and size of opening 2.38 mm.). Use a sieve 8 inches in diameter for cans under 3 pounds net weight, and a sieve 12 inches in diameter for larger containers. If the units of the product are "cupped" (for example, peaches in halves), turn over as quickly and gently as possible any units which may have fallen with cups up, then tilt the screen as much as possible without shifting of the units. After draining for two minutes from the time the product is poured on the screen, transfer contents to a tared dish and determine drained weight.

Using this method the two laboratories have determined drained weight in the cans of fruit indicated (Table 1).

No analysis of the data to determine differences in individual cans from the same lot has been made, except the general observation that variations in any three cans cut at either laboratory are usually as great as the variations noted in the entire six cans, cut three at each of the laboratories. There are too many factors affecting the drained weight of canned fruit to permit the preparation of duplicate cans, with any assurance that the drained weight of each can will be identical. Therefore the only significant check between two analysts is the average by each analyst of the analyses of a large number of cans with known put-in weights of fruit.

In view of the close agreement in relationship between put-in weight of fruit and drained weight, as shown by the above results on over 900 cans of fruit examined by two laboratories using the above method, it is recommended that this method be substituted in the present official method for the last clause in the second sentence of the first paragraph, all the last sentence of that paragraph, and the first two sentences in the second paragraph. While the collaborative work mentioned was done entirely on canned fruits, work in the Canned Food Section shows the method to be equally applicable to canned vegetables, with the exception of canned tomatoes. The 8-mesh screen is too fine for satisfactory results in draining the latter article, therefore it is further recommended that a statement be added to the official method showing that it is not applicable to canned tomatoes.

TABLE 1.—*Drained weight*

	CANNED FOOD SECTION		NAT. CANNERS ASS'N	
	NO. OF CANS	DRAINED WEIGHT—% OF PUT-IN WEIGHT	NO. OF CANS	DRAINED WEIGHT—% OF PUT-IN WEIGHT
Apricots, whole unpeeled	377	90.0	378	90.7
Cherries, Royal Anne	264	94.1	264	94.3
Peaches, Yellow Cling, halves	195	94.0	195	94.8
Peaches, Yellow Cling, sliced	47	93.0	48	94.2
Pears, Bartlett, halves	32	95.9	32	95.3

The Referee's Laboratory has undertaken further studies on the relationship between the alcohol-insoluble solids of canned peas and the observed maturity, in the field, of those peas before being canned. S. C. Rowe and L. M. Beacham of the Laboratory studied field conditions and made detailed observations of the maturity of Alaska variety peas being harvested for canning and obtained samples of the observed peas after canning, at thirteen plants located in Virginia, Maryland, Delaware, and Wisconsin.

After making arrangements with the canner for conducting the experiment, Rowe and Beacham observed the field from which peas were being harvested and noted the general appearance of vines and pods. A load of peas was then followed to the viner and a sample, as nearly representative as possible, was taken from the load. The pods in this sample were removed from the vines and were classified according to their apparent maturity, as follows:

F—Pods in which the peas had not developed sufficiently to give any peas large enough for canning.

M_1—Pods in which the peas had developed, but in which they were still too small to completely fill the pods.

TABLE 2.—*Relationship of pod maturity to alcohol-insoluble solids in canned Alaska peas*

CODE	F PODS	M_1 PODS	M_2 PODS	M_3 PODS	M_4 PODS	SIEVE SIZE	TOTAL	A.I.S.
			per cent				*per cent*	
WMPA	17	25.6	56.8	0.7	0	1	5.0	—
2						2	20.0	10.4
3						3	42.5	14.7
4						4	32.5	18.8
TCB 1–2	2.8	38.8	57.5	0.8	0	1–2	36.4	—
3						3	54.5	18.3
4						4–5	9.1	19.6
TCA 1–2	8.1	31.8	57.8	2.3	0	1–2	34.7	13.11
3						3	52.1	18.62
4						4–5	13.0	20.08
WVA 1	10.7	14.0	71.8	3.5	0	1	13.0	9.5
2						2	27.0	12.1
3						3	35.0	15.8
4						4	25.0	19.3
TCD 1–2	3.2	33.3	58.9	4.6	0	1–2	19.0	10.8
3						3	36.0	15.1
4						4–5	45.0	18.6
TCC 1–2	3.9	18.3	72.8	5.1	0	1–2	33.3	16.7
3						3	55.5	21.3
4						4–5	11.2	22.2
SAPR	20.7	20.0	51.3	8.0	0	Pod Run	100.0	13.6
WMPB 1	0	0	79.3	20.7	0	1	1.5	9.7
2						2	12.5	12.1
3						3	48.0	16.5
4						4	36.5	19.6
5						5	1.5	21.3
TCE 1–2	0	29.5	47.7	22.8	0	1–2	16.7	—
3						3	33.3	16.8
4						4–5	50.0	20.4
WBB	2.5	4.1	84.3	8.7	0.4	1	3.0	—
2						2	20.0	12.3
3						3	60.0	18.4
4						4	15.0	20.1
5						5	2.0	22.9
WMPC 1	1.3	6.6	66.9	24.7	0.5	1	1.0	10.0
2						2	12.5	14.5
3						3	46.5	19.4
4						4	39.0	21.3
5						5	1.0	23.3
LAPR	12.7	23.0	52.5	10.6	1.2	Pod Run	100.0	15.6
WWA	10.0	1.8	78.0	8.8	1.4	1	1.0	—
2						2	15.0	12.5
3						3	55.0	16.7
4						4	25.0	22.1
5						5	4.0	21.8
DAPR	4.5	4.2	59.6	30.1	1.6	Pod Run	100.0	19.8

TABLE 2.—*Relationship of pod maturity to alcohol-insoluble solids in canned Alaska peas—Continued*

CODE		F PODS	M₁ PODS	M₂ PODS	M₃ PODS	M₄ PODS	SIEVE SIZE	TOTAL	A.I.S.
				per cent				*per cent*	
TCF	1–2	1.3	3.2	47.9	45.0	2.6	1–2	14.4	11.8
	3						3	42.8	16.9
	4						4–5	42.8	21.0
KC	1–2	3.0	9.1	37.2	47.3	3.4	1–2	13.1	17.7
	3						3	34.8	21.5
	4						4	52.1	23.6
WSCA		0	3.3	62.3	30.8	3.7	1	1.0	—
	2						2	12.0	13.1
	3						3	50.0	17.0
	4						4	35.0	21.5
	5						5	2.0	23.5
KEPR		0.4	3.7	35.1	56.7	4.3	Pod Run	100.0	23.8
KFPR		0.6	4.1	38.6	52.2	4.5	Pod Run	100.0	23.7
FB	1–2	2.5	6.7	46.5	39.4	4.6	1–2	20.0	17.2
	3						3	50.0	22.4
	4						4–5	30.0	23.0
FD	1–2	3.5	8.8	29.4	52.7	5.7	1–2	20.4	18.2
	3						3	48.4	21.4
	4						4–5	31.2	22.9
FE	1–2	6.5	8.7	36.0	42.6	6.3	1–2	20.0	16.7
	3						3	50.0	21.0
	4						4–5	30.0	22.5
KB	1–2	1.6	7.3	38.9	45.5	6.7	1–2	12.0	17.6
	3						3	40.0	20.6
	4						4–5	48.0	22.6
LBPR		4.9	6.6	38.0	43.8	6.7	Pod Run	100.0	22.0
FC	1–2	2.2	8.7	39.9	41.8	7.4	1–2	22.8	14.4
	3						3	50.0	19.7
	4						4–5	27.2	22.6
KDPR		2.5	7.6	38.8	43.5	7.7	Pod Run	100.0	23.1
KA	1–2	2.4	7.0	47.8	34.8	8.0	1–2	6.8	14.1
	3						3	26.6	17.3
	4						4–5	66.6	22.0
WSCB		0.7	4.8	53.4	33.0	8.0	1	2.0	—
	2						2	12.0	13.5
	3						3	45.0	17.9
	4						4	40.0	21.5
	5						5	1.0	21.7
DCPR		6.0	8.0	30.0	47.1	8.9	Pod Run	100.0	20.6
WBA		0.6	2.3	30.2	54.8	12.1	1	2.0	—
	2						2	11.0	20.0
	3						3	55.0	22.2
	4						4	28.0	25.0
	5						5	4.0	26.4
LCPR		2.8	7.2	33.7	37.9	18.5	Pod Run	100.0	23.1
SBPR		1.5	3.2	30.7	43.7	20.8	Pod Run	100.0	22.4

TABLE 2.—*Relationship of pod maturity to alcohol-insoluble solids in canned Alaska peas—Continued*

CODE		F PODS	M_1 PODS	M_2 PODS	M_3 PODS	M_4 PODS	SIEVE SIZE	TOTAL	A.I.S.
					per cent				*per cent*
DBPR		8.4	3.9	25.8	41.1	20.8	Pod Run	100.0	22.4
LDPR		2.1	4.9	30.1	36.1	26.8	Pod Run	100.0	23.8
WELA		0	1.0	8.7	57.8	32.5	1	2.8	—
							2	1.4	—
	3						3	27.8	23.7
							4	1.4	—
	5						5	66.6	24.3
WKA		0	0.4	11.1	53.8	34.8	1	2.0	—
							2	10.0	—
	3						3	35.0	21.5
	4						4	35.0	21.4
	5						5	10.0	23.6
	6						6	8.0	24.5
FA	1–2	0.6	2.4	27.6	29.5	39.8	1–2	23.5	17.8
	3						3	53.0	21.7
	4						4–5	23.5	23.4

M_2—Pods in which the peas had developed sufficiently so that they were tight in the pod, but with shells still full of juice and brittle.

M_3—Pods in which the peas were tight but the shells thin (papery) and tough. Pods that had whitened and started to wrinkle slightly on the lower edge were included in this group.

M_4—Pods in which the peas were beginning to loosen because of shrinkage, with the shells thin (papery) and tough. Pods with yellow shells were also included in this group.

After the sample had been analyzed in this way, and knowledge obtained of the maturity of the peas in the load, the load was vined. These vined peas were kept separate in marked boxes, and a record was kept of the weight of the peas so obtained. In those plants where the peas were graded for size, the peas under observation were graded separately and a record kept of the weight of each sieve size resulting. The peas were then sent through the regular factory canning process, care being taken to keep them identified until they had passed through the closing machine. At the closing machine a representative sample of 12 cans of each sieve size, or 24 cans if the peas were ungraded for size, was taken and identified with code marks. These cans were then given the regular factory cook and cooling process.

This procedure was usually repeated at each plant several times, and thus samples from fields at several different stages of maturity were secured. A record was kept of other variable factors such as the time elapsing between cutting and vining, and between vining and canning,

but these did not show a significant effect upon the alcohol-insoluble solids content.

All the cans obtained in this way have not been analyzed for alcohol-insoluble solids, but all cans in some of the lots have been examined, and a preliminary test of at least 1 can from each lot has been made. As shown in Table 2, 10 of the 95 lots show an alcohol-insoluble solids content of more than 23.5 per cent. The lowest percentage of M_4 pods found in a sample having an alcohol-insoluble solids content of more than 23.5 per cent was in the case of KC-4, where 3.4 per cent M_4 pods were found in the unvined peas and the alcohol-insoluble solids content of the Sieve 4 peas was 23.6 per cent. Twenty-two of the lots had 3.4 per cent or more M_4 pods, while only 10 of the analyses on the corresponding canned peas showed an alcohol-insoluble solids content of 23.5 per cent or more.

The results from these analyses show that a definite relationship exists between the maturity of the peas used for canning and the alcohol-insoluble solids of the canned peas. While the alcohol-insoluble solids of the larger sieve sizes consistently ran higher than those of smaller sizes of the same lot, even in the large sieve sizes 23.5 per cent was exceeded only when a considerable number of mature pods were present. In lots that showed no pods of M_4 maturity, the alcohol-insoluble solids remained below 23.5 per cent, approaching this figure as the proportion of the M_1 and M_2 pods decreased and that of the M_3 pods increased. When a large per cent of M_3 pods was found with several per cent of M_4 pods, the alcohol-insoluble solids usually increased slightly beyond 23.5 per cent for the larger sieve sizes, and continued to increase slightly as more M_4 pods were noted. In many cases large quantities of M_4 pods were encountered where the alcohol-insoluble solids ran less than 23.5 per cent. In every case, however, where alcohol-insoluble solids of 23.5 per cent or more were observed, peas containing a preponderance of M_3 pods and considerable amounts of M_4 pods had been used for canning.

Table 3 shows the relatively close agreement in the alcohol-insoluble solids content of different cans from the same lot. These cans were taken consecutively, and at regular intervals, so that Can No. 1 represents the first, and Can No. 24, the last peas canned from the lot.

RECOMMENDATIONS[1]

It is recommended that the method for determining alcohol-insoluble material in canned peas, *This Journal*, **21**, 89 (1938), be clarified as follows:

(a) Change the title to read, "Alcohol-Insoluble Solids in Canned Peas and Canned Dried Peas."

(b) Change the 3rd sentence on p. 90 to read as follows: "Grind the drained peas in a food chopper until the cotyledons are reduced to a smooth homogeneous paste, stir, and weigh 20 g. of the ground material into a 600 cc. beaker."

TABLE 3.—*Alcohol-insoluble solids content of different cans from the same lot*

CAN NO.	SIEVE SIZE	ALCOHOL-INSOLUBLE SOLIDS
		per cent
1	1 & 2	12.86
2	1 & 2	13.27
3	1 & 2	12.98
4	1 & 2	12.82
5	1 & 2	12.92
6	1 & 2	12.88
7	1 & 2	13.46
8	1 & 2	13.13
9	1 & 2	13.28
10	1 & 2	13.11
11	1 & 2	13.33
12	1 & 2	13.31
13	1 & 2	12.95
14	1 & 2	12.91
15	1 & 2	12.77
16	1 & 2	13.07
17	1 & 2	13.30
18	1 & 2	13.24
19	1 & 2	13.26
20	1 & 2	13.00
21	1 & 2	13.05
22	1 & 2	13.42
23	1 & 2	13.17
24	1 & 2	13.07
1	3	18.54
2	3	18.35
3	3	18.60
4	3	18.56
5	3	18.67
6	3	18.42
7	3	18.64
8	3	18.81
9	3	18.41
10	3	18.63
11	3	18.44
12	3	18.90
13	3	18.74
14	3	18.60
15	3	18.60
16	3	18.90
17	3	18.76
18	3	18.84
19	3	18.47
20	3	18.55
21	3	18.54
22	3	18.78
23	3	18.39
24	3	18.80

TABLE 3.—*Alcohol-insoluble solids content of different cans*
from the same lot—Continued

CAN NO.	SIEVE SIZE	ALCOHOL-INSOLUBLE SOLIDS
		per cent
1	4	20.05
2	4	20.14
3	4	19.89
4	4	19.96
5	4	19.92
6	4	19.85
7	4	20.02
8	4	20.07
9	4	20.20
10	4	20.03
11	4	19.97
12	4	20.26
13	4	20.00
14	4	19.85
15	4	20.53
16	4	20.54
17	4	20.23
18	4	20.14
19	4	20.10
20	4	19.80
21	4	20.17
22	4	19.92
23	4	20.15
24	4	20.03

(c) Combine the first and second complete paragraphs on p. 90 to read as follows: "Fit into a Büchner funnel a filter paper of appropriate size, previously prepared by drying in a flat-bottomed dish for 2 hours at the temp. of boiling H_2O, covering with a tight-fitting cover, cooling in a desiccator, and weighing at once. Apply suction and transfer the contents of the beaker to the Büchner funnel in such a manner as not to run over the edge of the paper; suck dry and wash the material on the filter with 80% alcohol until the washings are clear and colorless."

CORRECTIONS

Vol. **22**, No. 1, *This Journal:*

Page 72, end of line 23, "10.2" should read " ±0.1." Line 24, "1.0" should read 1.0%."

Page 125, line 5 above heading "Discussion," "4X" should read "for X."

Page 195, line 10, "(8″×10″)" should read "(8″×1″)."

CONTRIBUTED PAPERS

IDENTIFICATION OF FLAVORING CONSTITUENTS IN COMMERCIAL FLAVORS

V. QUANTITATIVE DETERMINATION OF β-IONONE

By JOHN B. WILSON*

The preparation of ionone by Tiemann and Krueger is considered one of the outstanding triumphs of modern chemical research. It is discussed in detail in works on perfumery and essential oils (1) (2) since ionone was the original artificial violet perfume. Later, ionone was found to be a mixture of two isomeric ketones, differing in the position of the double bond. Tiemann and Krueger were really aiming to produce irone, a third isomer, which they had found to be the characteristic odorous constituent of orris root (3). Irone differs from both α- and β-ionone by the position of the double bond. The constitutional formulas attributed to the three compounds are given below.
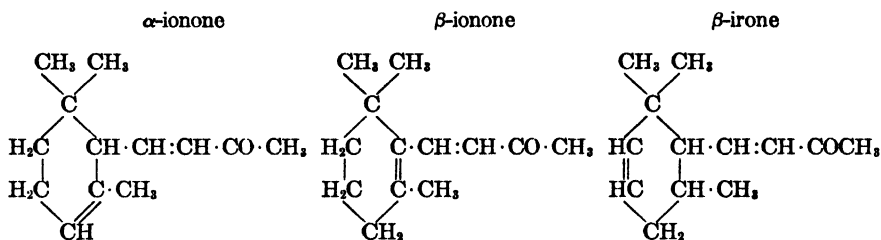
Besides the three compounds under discussion, fifteen other isomers having the formula $C_{13}H_{20}O$, including α-irone, iso-irone, and tuberone, are described in the literature (4), but none has a commercial importance approaching that of α- and β-ionone, which have been used extensively in the perfume industry for almost a half century.

Some years ago manufacturers began to add β-ionone to flavors because when diluted this chemical perfume gives an odor resembling that of raspberries. Raspberries are among the most easily manipulated fruits for use in the manufacture of flavors. The color of raspberries, especially the black varieties, is sufficiently intense to permit considerable dilution before it fades into insignificance. The flavor of raspberries, in spite of its delicacy, is quite characteristic, and it usually survives manipulations better than do the flavors of most other fruits. However, the addition of 1 or 2 mg. of β-ionone to 1 ounce of a so-called true fruit raspberry flavor will permit dilution to one gallon (128 fluid ounces) of bottler's or fountain sirup whereas only 30 fluid ounces would be possible without such addition. In the preparation of dry products, such as gelatin desserts, even

greater dilutions are possible because the evanescent fruit flavor may be lost before the product reaches the ultimate consumer, while the less volatile β-ionone remains to give an artificial raspberry flavor to the final product.

REVIEW OF LITERATURE

A review of the available literature brought to light a number of methods designed for use in the examination of commercial ionones for purity. Five derivatives of β-ionone are described in Beilstein (4). The oxime is an oil, while the oxime acetic acid and ketazine are described as crystalline substances melting at 103° and 105° C., respectively. The semicarbazone and thiosemicarbazone melt at 148°–149° C. and 158° C., respectively.

The procedures given by Tiemann (5) for preparation of the first four of the derivatives mentioned above are recommended for use in the commercial purification of ionone and are designed for this purpose. Tiemann (5) also gives directions for the preparation of the p-bromophenylhydrazone, oxyionolactone, and other compounds of value in separating the isomers commercially.

A number of other procedures recommended especially for ionone by various authors were tried out, as well as some reagents frequently used for the detection and determination of aldehydes and ketones. The results are given below.

EXPERIMENTAL

The purity of the β-ionone used in the early experiments was determined by the method of Hendrikse and Reclaire (6) and found to be 93.2 per cent and 92.96 per cent in two duplicate determinations. Two duplicate determinations by the method of Radcliffe and Swan (7) for total ketones gave 91.88 per cent and 91.66 per cent, respectively. These procedures are applicable to comparatively pure commercial ionones, but not to quantities of 100 mg. or less in complex mixtures.

The most promising method found in the literature, from the point of view of determining moderate quantities of ionone, was that of S. Ito (8). This method, which is recommended for use on about 200 mg. of sample, precipitates the ionone as semicarbazone. It was applied by the writer to solutions containing various quantities of β-ionone, with the results given in Table 1. As Ito's method is not readily available in English, it is recorded here. The free translation given was prepared by the writer from a literal translation made by Saburo Katsura from the original article in Japanese.

Dissolve a weighed portion of the ionone in a small quantity of alcohol, add a saturated water solution of semicarbazide HCl containing three times the quantity necessary for combination with the ionone believed to be present. Add more alcohol if necessary to obtain a clear solution. Permit the mixture to stand for 24 hours.

Add 200 cc. of cold water, place in a refrigerator, and after 24 hours filter, dry, and weigh. (Ito used .15–.40 gram of sample in testing the procedure.)

The semicarbazone formed is a white sticky precipitate that filters readily but melts even on drying at 80° C. in vacuo in spite of a recorded melting point of 148°–149° C. On cooling the melted substance gives the appearance of rosin. The data (Table 1) show a good recovery for quantities approaching 200 mg., but recovery falls off rapidly as the quantity of ionone is decreased.

TABLE 1.—*Determination of β-ionone by Ito's method*

EXPERIMENT NO.	β-IONONE PRESENT	SEMICARBAZONE	β-IONONE FOUND	RECOVERY
	gram	*gram*	*gram*	*per cent*
111	0.2307	0.2814	0.2170	94.06
112	0.1355	0.1596	0.1231	90.85
113	0.0757	0.0850	0.0655	86.53
114	0.0372	0.0382	0.0295	79.30
115	0.0100	0.0043	0.0033	33.00

The writer also attempted to prepare crystalline β-ionone semicarbazone as directed by Veibel (9), Tiemann and Krueger (10), and Chuit (11), but was not successful.

The thiosemicarbazone formed when the determinations of purity were made by the Radcliffe and Swan method (7) was recovered by evaporation of the carbon bisulfide and found to be a thick oily liquid that did not crystallize despite all efforts to cause it to do so. A portion of the compound was dissolved in alcohol, but it did not crystallize even when kept in the refrigerator for two weeks after dilution with water. No crystalline β-ionone thiosemicarbazone was obtained when the procedure recommended by Chuit (11) was used, and when silver nitrate was added to the alcohol solution of the salt as recommended by Neuberg and Neimann (12) for precipitation of metallic salts of thiosemicarbazones of aldehydes and ketones, the white precipitate that formed at once turned dark brown in a few moments, probably due to the reducing effect of the double bond in the ionone.

Tiemann (5) states that when concentrated solutions of p-bromophenylhydrazine and β-ionone in acetic acid are mixed, crystals of β-ionone-p-bromophenylhydrazone separate out. The writer added a solution of 0.2 gram of p-bromophenylhydrazine hydrochloride and 0.2 gram of sodium acetate in 10 cc. of acetic acid to 100 mg. of β-ionone in 5 cc. of acetic acid. As no precipitate had formed after 48 hours, 15 cc. of water was added to the solution, which then became cloudy and began to deposit crystals within 15 minutes. Gradually, 40 cc. of water was added with perceptible increases in the quantity of crystals formed. After the solution had stood 48 hours the crystals were filtered off, washed with

water, dried at 100° C., and weighed. The weight of the crystals corresponded to 58.4 mg. of β-ionone, showing a recovery of 62.8 per cent based on the purity of 93 per cent β-ionone in the sample taken. The crystals were dark in color.

As these reagents did not give acceptable results, the writer tried a large number of reagents known to have produced crystalline precipitates with one or more aldehydes or ketones, and at the same time made a blank determination to obviate errors due to insolubility of the reagent under the conditions obtaining.

Para-nitrophenylhydrazine, methylphenylhydrazine, diphenylhydrazine, benzylphenylhydrazine, betanaphthyl-hydrazine, hydroxylamine, thiosemicarbazide, dimethylhydroresorcinol, and 4-4-diphenylsemicarbazide, when used as directed in "Analyse Konstitutionsermittlung Organisches Verbindungen," by Hans Meyer, yielded with β-ionone oily or resinous precipitates ill-suited to quantitative work.

β-ionone gave a small amount of highly colored crystals with 2-4 dinitrophenylhydrazine (13) and with semioxamizid white crystals similar to those obtained in the blank.

META-NITROBENZHYDRAZIDE AS A PRECIPITANT FOR β-IONONE

Finally the writer turned to *m*-nitrobenzhydrazide, which was recommended as a precipitant for aldehydes and ketones by Curtius and his coworkers (14). A quantity (0.3 gram) of *m*-nitrobenzhydrazide was dissolved in 20 cc. of alcohol (1+1), and 0.23 gram of β-ionone dissolved in 10 cc. of alcohol was added. After two days it was found that crystals had formed in the liquid. About 30 cc. of water was added a little at a time until the solution became cloudy. The flask was stoppered and again set aside for two days, at the end of which time a perceptible increase in the quantity of precipitate was noted. Some of the precipitate was crystalline and some oily in appearance.

Encouraged by the results of this experiment the writer added 0.3 gram of *m*-nitrobenzhydrazide dissolved in 20 cc. of alcohol (1+1) to 10 cc. of alcohol containing 200 mg. of β-ionone. The flask was stoppered, and after three days the well-formed crystals were filtered off, washed with 18 cc. of dilute alcohol (3+4), then with 100 cc. of water, dried at 100° C., and weighed. The precipitate amounted to 0.1366 gram, equivalent to 73.9 mg. of β-ionone, or about 37 per cent of the quantity taken. Several more precipitations were made, and it was found that by diluting the solution with water the yield could be increased to 87 per cent, but in every case after dilution some oily matter was present in the precipitate.

As directed by Curtius and Reinke (15) for precipitation of aldehydes and ketones with *m*-nitrobenzhydrazide, 10 cc. of alcohol containing 100 mg. of β-ionone was diluted to 350 cc. with water, and 0.3 gram of the reagent dissolved in 30 cc. of alcohol (2+1) was added. The mixture was

refluxed on the steam bath for 30 minutes, cooled, and after being allowed to stand 3 days it was filtered. The precipitate was washed, dried, and found to weigh 89 mg., equivalent to 48 mg. of β-ionone. The filtration was very slow, and as the precipitate adhered to the flask in an alarming manner, it was washed out with ether, dried, and found to weigh 22 mg., corresponding to 12 mg. of β-ionone. Other quantities of ionone yielded similar recoveries.

A variation of the above procedure suggested by S. Reznek of this Administration was also tried. The *m*-nitrobenzhydrazide was dissolved in acetic acid (1+3) and added to 100 cc. of 5 per cent alcohol containing 50.3 mg. of β-ionone. After standing overnight the mixture was quite cloudy. After being shaken violently for 5 minutes, it was found that the precipitate was no longer attached to the sides and bottom of the flask but floated about in the otherwise clear solution. This precipitate, as well as two others obtained from other quantities of β-ionone, was filtered, dried, and weighed. The results are given in Table 2.

TABLE 2.—*Determination of β-ionone as m-nitrobenzhydrazide as directed by S. Reznek*

EXPERIMENT NO.	β-IONONE PRESENT	WT. OF PRECIPITATE	β-IONONE FOUND	RECOVERY
	mg.	*mg.*	*mg.*	*per cent*
186	50.3	81	43.8	87
247	50.0	77	41.7	83
248	20.0	30	16.2	81

It was found that precipitation occurred in the reagent after a few days, which caused doubt as to the composition of the precipitates obtained above. This factor, in conjunction with the low yields, caused the writer to abandon work on this procedure.

As a preliminary step to increasing the yield of β-ionone-*m*-nitrobenzhydrazide obtained by the first procedure tried, experiments were conducted to ascertain the solubility of this salt in various strengths of alcohol. A weighed quantity of substance was placed in a small Erlenmeyer flask, dissolved in a mixture of alcohol and water by warming on the steam bath, stoppered, and set aside for about 1 hour to cool. Some of the mixture was left in the room, and some was placed in an electric refrigerator kept at about 10° C. After 24 hours the precipitate was filtered, washed twice with 5 cc. portions of dilute alcohol (30 cc. of 95 per cent alcohol made up to 100 cc. with water), dried at 100° C., and weighed. The results are given in Table 3.

The data in Table 3 show that cold 30 per cent alcohol has little solvent action upon β-ionone-*m*-nitrobenzhydrazide, especially in the presence of excess reagent and acetic acid, and is therefore a suitable medium for precipitating and washing this compound.

After a considerable amount of experimentation with a view to obtaining as large a yield as possible and at the same time obtaining a crystalline precipitate containing as little oily material as possible, a procedure for precipitating β-ionone as m-nitrobenzhydrazide was worked out as given below.

TABLE 3.—*Solubility of β-ionone-m-nitrobenzhydrazide in alcohol of various strengths*

EXPERIMENT NO.	ALCOHOL USED		TEMPERATURE	β-IONONE-M-NITROBENZHYDRAZIDE			SOLUBILITY
	VOLUME	STRENGTH		TAKEN	RECOVERED	DISSOLVED	
	cc.	*per cent*		*mg.*	*mg.*	*mg.*	*mg. per 100 cc.*
295	12.0	55.4	Room	102.4	81.4	21.0	175.0
299	19.0	45.0	Room	101.8	94.7	7.1	37.4
298	17.0	39.1	Room	100.0	95.0	5.0	29.4
301	14.0	47.5	Room	102.9	92.3	10.6	75.7
303	20.0	47.5	Room	106.2	98.5	7.7	38.5
296	12.0	55.0	Refriger.	113.0	102.0	11.0	91.7
302	14.0	47.5	Refriger.	100.9	95.8	5.1	36.4
304	20.0	47.5	Refriger.	99.6	93.6	6.0	30.0
300	19.0	45.0	Refriger.	114.5	108.8	5.7	30.0
318*	16.0	32.7	Refriger.	51.5	49.8	1.7	10.6
323*	15.5	30.6	Refriger.	50.0	50.0	0.0	0.0
311*	15.0	30.0	Refirger.	115.8	113.8	2.0	13.3
310*	15.0	30.0	Refriger.	113.5	112.9	0.6	4.0
309*	15.0	30.0	Refriger.	102.5	101.3	1.2	8.0

* 0.05 of m-nitrobenzhydrazide and 0.2 cc. acetic acid added.

QUANTITATIVE PRECIPITATION OF β-IONONE AS M-NITROBENZHYDRAZIDE

Place 5 cc. of alcohol containing 10–100 mg. of β-ionone in a 125 cc. conical flask. Add 95–100 mg. of solid m-nitrobenzhydrazide and dissolve by warming the solution on the steam bath, taking precautions to prevent loss of alcohol through evaporation. Add 5 cc. of water, and if the solution becomes cloudy, warm until clear. Remove the solution from the steam bath, add 0.2 cc. of glacial acetic acid, stopper the flask lightly, and place upon a wooden surface to prevent too rapid cooling. If about 20 mg. or more of β-ionone is present, crystals will begin to form within 30 minutes after the contents of the flask have reached room temperature. Let stand in the room for at least 2 hours (overnight does no harm) and add 5 cc. of water dropwise, mixing the solution continuously during the addition by rotating the flask. Stopper, let stand in the room for at least 1 hour, and place in the re-frigerator overnight or up to 48 hours. Filter through a No. 4 sintered glass crucible, wash with 30 cc. of cold 30% alcohol, using a wet policeman to remove precipitate adhering to the flask, and dry at 100° C. Weight of precipitate multiplied by 0.541 gives the corresponding weight of β-ionone.

A number of commercial β-ionones were purchased on the open market, and duplicate ionone determinations were made by the above procedure, 100 mg. of sample and 100 mg. of the reagent being used. In addition, determinations of total ketones as ionone were made by the Radcliffe and Swan method (7). The results are given in Table 4.

TABLE 4.—*Ionone and total ketones, as ionone, in commercial β-ionones*

SAMPLE NO.	IONONE	TOTAL KETONES AS IONONE
	per cent	*per cent*
1	94.68	93.20
	93.80	92.96
2	96.14	100.00
	95.71	
3	95.19	99.19
	94.88	
4	85.04	86.83
	84.08	

To establish the composition of the precipitate, four 5 cc. portions of alcoholic solution containing 50 mg. of a commercial β-ionone were precipitated in the usual manner and filtered upon Gooch crucibles. After they had been dried and weighed, the precipitate and asbestos were mixed with prepared copper oxide and transferred quantitatively to the combustion boat of an apparatus for the determination of nitrogen by the Dumas method. The results are given in Table 5.

TABLE 5.—*Determination of nitrogen on β-ionone-m-nitrobenzhydrazide precipitates by the Dumas method*

NO.	β-IONONE	PRECIPITATE	β-IONONE		NITROGEN	
	mg.	*mg.*	*mg.*	*per cent*	*mg.*	*per cent*
452	50	82.4	44.9	89.8	9.89	11.92
454	50	83.6	45.2	90.4	10.06	12.03
456	50	86.7	46.9	93.8	10.79	12.44
458	50	92.5	50.0	100.0	10.81	11.69
Theory	50	92.5	50.0	100.0	10.94	11.83

The data in Table 5 show that the precipitate has the composition expected for β-ionone-m-nitrobenzhydrazide.

To test the applicability of the method to other quantities of β-ionone, a series of solutions was prepared to contain varying quantities of substance. For the stock solution 6.0035 grams of β-ionone was dissolved in 95 per cent alcohol and diluted to 300 cc. with the same solvent. Various quantities of the stock solution were diluted with alcohol to 100 cc., and ionone was determined by the proposed method. The data are given in Table 6.

The data in Table 6 indicate that the accuracy of the proposed method for β-ionone is substantially the same for quantities ranging from 10 to 100 mg. in 5 cc. of alcohol solution.

The low solubility of β-ionone-m-nitrobenzhydrazide in 30 per cent alcohol, in which solvent the precipitation was made, and the uniformity of the ionone content found when such widely different quantities of

ionone were used for the precipitation lead to the conclusion that the commercial ionone used for these experiments actually contains 96 per cent of ionone and 4 per cent of impurities. The material used here was from Sample 2, reported in Table 4 as showing 96.14 per cent and 95.71 per cent of β-ionone by the recommended procedure and 100 per cent total ketones as ionone by the Radcliffe and Swan method. No evidence has been obtained as to the composition of the impurities present in this product.

TABLE 6.—*β-ionone in stock solution and its varying dilutions*

SOLUTION	VOLUME OF STOCK DILUTED TO 100 CC.	β-IONONE IN 5 CC. ALIQUOT	PRECIPITATE	β-IONONE	RECOVERY
	cc.	*mg.*	*mg.*	*mg.*	*per cent*
A	100	100	175.4	94.9	94.9
B	50	50	89.2	48.2	96.4
C	40	40	70.4	38.1	95.2
D	30	30	53.0	28.7	95.7
E	20	20	35.6	19.3	96.5
F	10	10	18.0	9.7	97.0

When applied to a number of commercial products for use in violet perfumes, about 1 gram was dissolved in alcohol and diluted to 100 cc. with the same solvent, and 5 cc. was treated as described above. Total ketones were also determined by the method of Radcliffe and Swan, a 1 gram sample and about 1 gram of thiosemicarbazide being used. The β-ionone found in these products, which from the standpoint of their use may well be grouped under "imitation violet oil," is given in Table 7.

TABLE 7.—*β-ionone and total ketone content of imitation violet oils*

MANUFACTURER	β-IONONE	KETONES AS IONONE
	per cent	*per cent*
A	81.51	85.19
	80.10	
B	73.68	71.58
	73.56	
C	64.40	63.16
	63.96	
D	52.06	

As many raspberry flavors, especially those of the so-called "True Fruit Type," contain a base of concentrated raspberry juice or alcoholic extract of fresh or dried raspberries to which may be added distillates from the berries and possibly small quantities of synthetic ingredients, the procedure of steam distillation was applied as a means of separating any β-ionone from the commercial flavor. The procedure of steam dis-

tillation was applied successfully in Part II of this series (16) and in studies of the volatile constituents of fruits (17) (18) (19).

Since the publication of the writer's paper in 1932, the advent of the standardized ground-glass joint has made it possible to improve the apparatus used for steam distillation by substituting all glass equipment of standard dimensions, which is listed below.

As previous experiments had shown that the procedure for aldehydes and ketones described in Part II of this series was not applicable to β-ionone, it was thought possible that after steam distillation the ionone could be extracted with a volatile solvent, held back by the reagent during evaporation of the solvent, and afterwards recrystallized or re-precipitated in the same manner as described above for quantitative precipitation of β-ionone. After considerable experimentation the procedure given below was devised. Both petroleum ether and sulfuric ether were tried for the extraction; the number of extractions needed were subjected to test; and the time of standing varied within certain limits.

β-IONONE IN RASPBERRY FLAVORS

APPARATUS

(a) *Steam generator filled with water.*—An oil can holding 1 gallon will serve the purpose.

(b) *Distillation flask.*—Round-bottomed boiling flask having interchangeable ground-glass connection 24/40, capacity about twice the volume of sample to be used.

(c) *Still head.*—Adapter, 75° angle, with interchangeable male connections 24/40 at bottom and side and female connection 14/35 at top, with side arm lengthened and bent to fit vertical condenser (like Ace Glass Co. No. 1180).

(d) *Spray tube.*—Adapter, for use with Woulff bottles equipped with interchangeable ground-glass connection, aeration tube with connection 14/35, holes in bulb approximately 2 mm. in a diameter, length of tubing such that when the apparatus is set up, the bulb is situated not more than 20 mm. above the bottom of the distilling flask (like Ace Glass Co. 1300-C).

(e) *Condenser.*—Coil type with interchangeable female connection 24/40 at top with 250 to 300 mm. jacket and outlet tube lengthened to about 200 mm. to reach bottom of receiving flask.

(f) *Receiving flask.*—Conical flask of 500 cc. capacity.

REAGENTS

(a) *Ethyl ether.*—Containing practically no alcohol.

(b) *M-nitrobenzhydrazide.*—Obtainable from Eastman Kodak Co.

(c) *Glacial acetic acid.*—Reagent grade.

(d) *Alcohol.*—95% by volume.

(e) *Dilute alcohol.*—Place 30 cc. of alcohol in a 100 cc. volumetric flask and dilute to the mark with water. Keep in a refrigerator.

PROCEDURE

Place 250–1000 cc. of sample (which should contain not more than 100 mg. of β-ionone) in the distilling flask and connect with the apparatus. Add enough water to the receiving flask to just cover the outlet of the condenser. Heat the sample

nearly to boiling on an asbestos mat with a flame or by immersing it in a boiling water bath. As soon as the sample has reached the temperature of the bath or has just begun to boil, connect with the steam generator and pass a rapid current of steam through the sample until 300–500 cc. of distillate has been collected.

Add sufficient water to the distillate to reduce the alcohol content to about 10 per cent or less and transfer to a large separatory funnel. Add 150–200 cc. of ether, depending upon the volume of solution, so that about 100 cc. will be obtained upon separation. Shake thoroughly and separate. Transfer the ether solution to a 125 cc. conical flask containing 95–100 mg. of *m*-nitrobenzhydrazide. Add 0.2 cc. of acetic acid and dissolve the solid reagent by stirring and breaking up lumps with a glass rod, warming if necessary to complete the solution. Permit the mixture to stand for about 1 hour and evaporate on the steam bath to about 10 cc., passing a current of air into the flask to hasten the evaporation and keep down the temperature. In the meantime make a second extraction of the distillate, using 100 cc. of ether. Add the separated ether solution to the flask containing the residue from the first ether extract and after allowing to stand about 15 minutes evaporate to 10 cc. as before. In a similar manner make a third extract, using 100 cc. of ether, add to the flask, and evaporate as before until only 1–3 cc. of watery liquid and perhaps some oily residue remain.

While the flask is still warm, add 5 cc. of alcohol from a pipet, allowing the liquid to wash down the sides of the flask, and dissolve the residue completely by warming on the steam bath, protecting the liquid against loss by evaporation. Add 5 cc. of water and warm if necessary to obtain a clear solution. Add 0.2 cc. of acetic acid, close with a cork stopper, and place the flask on a wooden surface to prevent too rapid cooling.

After 2 hours add 5 cc. of water dropwise, mixing the liquid by continuously rotating the flask, stopper, and keep at room temperature for at least 1 hour (overnight does no harm), then place in the refrigerator overnight or up to 48 hours.

Filter on a fritted glass crucible of porosity 4 and wash with about 30 cc. of dilute alcohol. Dry in a vacuum over at 70° C. and weigh. Wt. of ppt. × 0.541 = β-ionone.

This procedure was applied to the same diluted solutions that were used for testing the precipitation method and reported in Table 6. In each determination 5 cc. of the solution of β-ionone was added to 20 cc. of alcohol and 225 cc. of water in the steam distillation apparatus, and the procedure was carried out as described above. The results are given in Table 8.

TABLE 8.—*Recoveries of β-ionone by the proposed method*

SOLUTION	PRESENT	FOUND BY DETERMINATION				
		1	2	3	AV.	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*
A	100	94.7	96.0	96.8	95.8	95.8
B	50	47.0	47.5	47.8	47.4	94.8
C	40	37.9	38.1	38.4	38.1	95.2
D	30	28.5	28.7	28.8	28.7	95.7
E	20	18.8	18.9	19.0	18.9	94.5
F	10	9.4	9.0	9.5	9.3	93.0

The method was also applied to a number of commercial samples of products, labeled "True Fruit Raspberry," and while several products were found to contain no ionone, in a number of other cases the following quantities were determined: 50, 51, 72, 75, 77, 120, 132, and 160 mg. of β-ionone per liter of flavor. For several samples of both red and black raspberries negative results were obtained in every case. A detailed description of the procedure used is given in Part VI of this series, which follows this paper. As m-nitrobenzhydrazide combines with a number of aldehydes and ketones, precipitates obtained by the methods given here should be identified by the procedure given in Part VI.

SUMMARY

Several procedures recommended by various authors for the determination of ionone were tried out and found to give unsatisfactory results. A number of procedures involving the use of 14 different reagents recommended for the precipitation of aldehydes or ketones were then tried, and it was found that one reagent, m-nitrobenzhydrazide, gave a precipitate with β-ionone that appeared to be suited to its quantitative determination. Several procedures recommended for the application of this reagent to aldehydes and ketones were subjected to test and the most suitable one selected. A method was then developed for the quantitative determination of β-ionone as m-nitrobenzhydrazide and found to be accurate for quantities ranging from 10 to 100 mg. of β-ionone. The writer expects to apply this procedure to other similar ketones as a possible means of their identification.

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IDENTIFICATION OF FLAVORING CONSTITUENTS OF COMMERCIAL FLAVORS

VI. IDENTIFICATION OF β-IONONE AS M-NITROBENZHYDRAZIDE

By John B. Wilson and George L. Keenan*

Part V (1) of this series of papers deals with the quantitative determination of β-ionone. The reagent used is m-nitrobenzhydrazide, which was recommended as a precipitant for aldehydes and ketones by Curtius (2) and his co-workers, and was used for the quantitative determination of vanillin by Hanus (3). However, the writers did not consider the formation of a precipitate as sufficient evidence of the presence of β-ionone in commercial flavors, and wished to obtain a positive means of identifying β-ionone in products of unknown composition. To accomplish this end they had recourse to the immersion method used for the identification of certain aldehydes and ketones and reported in Part I of this series (4).

The procedure employed in Part I could not be used for preparation of a derivative since semicarbazide had been shown unsuitable for this purpose, as reported in Part V. However, the precipitate of β-ionone m-nitrobenzhydrazide that was found (Part V) to serve so well as a means of determining β-ionone, appeared to be crystalline to the naked eye. Accordingly, a quantity of the substance was prepared for microscopic study.

PREPARATION OF β-IONONE-M-NITROBENZHYDRAZIDE

After several experiments it was found that well crystallized β-ionone-m-nitrobenzhydrazide can be prepared in the following manner:

Place about 500 mg. of β-ionone and 600 mg. of m-nitrobenzhydrazide in a 200 cc. conical flask, add 50 cc. of alcohol, and dissolve by warming on the steam bath. When all the hydrazide is in solution, add 50 cc. of hot water, and continue the heating on the steam bath for about 5 minutes. Stopper the flask and set aside for about 20 minutes, then add 2 cc. of acetic acid and let stand overnight at room temperature and then in the refrigerator for 5–6 hours. Filter through a fritted glass crucible of No. 4 porosity, wash with several portions of 30 per cent alcohol totaling about 50 cc., and dry in vacuo at 70° C.

β-ionone-m-nitrobenzhydrazide made in this manner was found to be well suited to microscopic identification. When nitrogen was determined by the Dumas method the results obtained were 13.20, 13.27, and 13.24 per cent, which is somewhat higher than the theoretical result, 11.83 per cent.

This material was examined microscopically by the method described in Part I (4) and its properties are described below.

OPTICAL CRYSTALLOGRAPHIC PROPERTIES OF β-IONONE-*m*-NITROBENZHYDRAZIDE

To the naked eye, this substance in mass has a yellowish color. When examined in ordinary light under the microscope, the material is essentially colorless and crystallizes in thin, rod-like plates, many of them having lath-like or frayed ends, some of them having a six-sided outline. In parallel polarized light (crossed nicols), the extinction is parallel and the sign of elongation negative. The refractive indices as determined by the immerson method were the minimum and maximum values, these being $n_\alpha = 1.548$, invariably shown on the elongated fragments when their long dimension is parallel to the vibration plane of the lower nicol (lengthwise), and $n_\gamma = 1.648$ usually shown on the elongated fragments when their long dimension is at right angles to the vibration plane of the lower nicol (crosswise).

To identify β-ionone in a food product, steam distil a suitable sample of the food. Extract the distillate with ether and follow the procedure for the determination of β-ionone as given in Part V of this series. If a precipitate is obtained, examine it microscopically by the immersion method. If the precipitated material consists of oily matter mixed with crystalline matter, place the fritted glass crucible in a Gooch holder attached to a suction flask. By means of a wire support a test tube within the suction flask in such a manner as to catch any liquid that may pass through the crucible. Add about 5 cc. of petroleum ether, cover the crucible, and let stand for about 5 minutes. Turn on the suction just long enough to carry through any of the solvent that may remain in the crucible. Transfer the petroleum ether solution to a small beaker and allow it to evaporate spontaneously. Repeat several times until no more soluble matter is obtained by the extraction. Examine the remaining contents of the crucible and the several residues microscopically for crystals of β-ionone-*m*-nitrobenzhydrazide.

This procedure was applied to a number of commercial raspberry flavors and β-ionone was identified as a constituent. Subsequently, two of the manufacturers admitted that this substance had been added to their products. Several other commercial raspberry flavors were examined and no β-ionone-*m*-nitrobenzhydrazide was found in the insoluble matter.

In order to establish the presence or absence of β-ionone as a constituent of raspberries, a number of samples were examined. Two one quart samples of red raspberries weighing 668 grams and 632 grams, respectively, and two one quart samples of black raspberries weighing 615 grams and 654 grams, respectively, were examined by the recommended procedure, and no crystalline material whatsoever was found when the insoluble matter obtained was examined microscopically.

Larger samples were used for the next experiments. Three 3 kg. portions of frozen pack red raspberries (3+1) totaling 15 pounds of the berries were steam distilled. The steam distillates were extracted with ether in rotation, and the ether extracts were evaporated in a small flask containing 100 mg. of *m*-nitrobenzhydrazide, which was dissolved in the first portion of ether extract by warming slightly, and 0.2 cc. of acetic acid was added. The evaporation was carried out on a steam bath while a current of air played upon the surface of the liquid, the surface of the flask being cold to the touch at all times. When the solution had been evaporated to about 10 cc. the second portion of ether was added, allowed

to stand in contact with the reagent 15–20 minutes, then evaporated as before. This process was repeated until all the ether extracts had been evaporated in the same flask after being left in contact with the reagent long enough for the formation of the *m*-nitrobenzhydrazide of any β-ionone that might have been present in the distillate. The final evaporation was continued until only 2–3 cc. of watery liquid and some oily drops remained in the flask. The residue had the odor of raspberries, but no odor of β-ionone could be detected. The residue was then dissolved in 5 cc. of 95 per cent alcohol, and 5 cc. of water and 0.2 cc. of acetic acid were added. The solution stood overnight at room temperature to permit β-ionone-*m*-nitrobenzhydrazide (if present) to crystallize. The next day 5 cc. more water was added, and the solution was allowed to remain in the refrigerator for about 48 hours. It was then removed, filtered, washed with 30 per cent alcohol, dried, and weighed.

When submitted to microscopic examination, no crystals of β-ionone-*m*-nitrobenzhydrazide were present, nor could any other crystalline material of any kind be found in the crucible. The crucible contained only a minute quantity of oily or gummy material, which had partially soaked into the fritted glass of the crucible and still retained the odor of the berries, which odor was also apparent in the filtrate. When washed with petroleum ether, none of the matter in the crucible dissolved or came through the crucible.

The test was repeated on another sample of red raspberries consisting of four portions aggregating 13 kg. of frozen pack red raspberries with sugar, containing not less than 24 pounds of red raspberries. Here again no crystals of β-ionone-*m*-nitrobenzhydrazide occurred, nor was any other crystalline material found.

A 12.2 kg. sample of frozen pack black raspberries with sugar, containing not less than 22 pounds of black raspberries, and a 10 kg. sample of frozen pack black raspberries (3+1) seedless, containing not less than 16 pounds of fruit, were examined in the same manner. The microscope showed that the insoluble matter contained no crystals of β-ionone-*m*-nitrobenzhydrazide nor any other crystalline material whatsoever.

In the opinion of the writers, these data constitute a negative result for the test for β-ionone in either red or black raspberries.

SUMMARY

A quantity of β-ionone-*m*-nitrobenzhydrazide was prepared, and its optical crystallographic properties were determined. Three samples each of red and black raspberries, consisting of as much as 24 pounds and 22 pounds, respectively, were examined, and no β-ionone was found to be present.

In certain cases samples of commercial so-called true fruit raspberry flavors were found to contain β-ionone, and in other cases none was found.

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IDENTIFICATION OF FLAVORING CONSTITUENTS IN COMMERCIAL FLAVORS

VII. QUANTITATIVE DETERMINATION OF COUMARIN

By JOHN B. WILSON*

During the past few years the physiological effects of coumarin and its degradation products in cured hay gave rise to the need for a precise method for the determination of coumarin for the use of the plant physiologists who have been conducting breeding experiments on grasses with a view to eliminating this constituent from this type of forage. This need has now been met by the work of several investigators. Clayton and Larmour (1) appear to have been the first to suggest the use of the color produced by coupling coumarin with diazo-*p*-nitroaniline in alkaline solution for this purpose, although the original work on this condensation was reported by Mitchell (2), and the same color used by Chakravati (3).

After a thorough investigation Roberts and Link (4) published a method for the determination of coumarin, melilotic acid, and coumaric acid in plant tissue. Duncan and Dustman, whose steam distillation method for coumarin (5) was published several years ago, have revised their procedure and are now (6) substituting a modified form of the Roberts and Link procedure for the actual colorimetric measurement of coumarin for their previous color test and recommend the distillation method for the determination of coumarin in vanilla.

The writer desired to find a quick method for the determination of coumarin in imitation vanilla, which according to trade practice may contain from 0.05 to 0.20 per cent of coumarin, with vanillin in proportions ranging from 0.10 to 0.70 per cent, or even more on occasion. As the official method for coumarin (7) is somewhat tedious, and as Duncan and Dustman (6) have shown that at least two and sometimes three steam distillations under reduced pressure are needed to completely recover added coumarin from vanilla products, the writer sought to use the colorimetric procedure without distillation.

EXPERIMENTAL

Solutions of vanillin and coumarin were prepared of such strength that the colorimetric test could be applied to different quantities of each,

either alone or mixed in varying proportions. The test of Duncan and Dustman (6) was applied, and it was found that both substances give a color to the naked eye. However, with the assistance of P. A. Clifford of the U. S. Food and Drug Administration the writer found that when the several solutions were examined in a spectrophotometer designed by Clifford and Brice and the proper filter used, coumarin could be determined even in the presence of vanillin. It was also found that filter No. 49 gave the best spread over a workable range with coumarin and at the same time registered the least interference from vanillin.

As it had been recorded by Duncan and Dustman that vanillin interferes with this colorimetric determination, the first experiments were made with increasing quantities of coumarin and with a quantity of vanillin five times as great as the maximum quantity of coumarin. This is a ratio frequently found between these two flavoring ingredients in imitation vanilla. The results of this experiment are given in Table 1.

TABLE 1.—*Measurement of color intensity of coumarindiazo-p-nitraniline*

VANILLIN IN 50 CC.	COUMARIN IN 50 CC.	READING FILTER NO. 49 AFTER—	
		2 HRS.	24 HRS.
mg.	*mg.*	*mm.*	*mm.*
3	0.0	3.5	9.8
3	0.1	25.5	31.5
3	0.2	48.5	54.8
3	0.3	72.2	78.3
3	0.4	94.4	99.4
3	0.5	125.0	128.6
3	0.6	off scale	

The results given are in all cases the average of 5–10 individual readings.

The data in Table 1 indicate that quantities of coumarin up to 0.5 mg. in the aliquot used may be determined with the spectrophotometer even in the presence of vanillin and that the color deepens somewhat on standing for 24 hours.

The next experiment was to ascertain the effect upon solutions of the same coumarin content of varying the proportion of vanillin. As 0.3 mg. of coumarin appears to be the mean of the workable range, this quantity was used, and the vanillin was varied from 0.0 to 10.0 mg., which last quantity is 33 times the quantity of coumarin present. The data are given in Table 2.

As heliotropin (piperonal) is sometimes used in imitation vanilla, the effect of this substance was tried in a similar manner (Table 3).

These two experiments show that neither vanillin nor heliotropin has a deleterious effect upon the determination of coumarin by the procedure recommended.

TABLE 2.—*Effect of varying proportions of vanillin on coumarin determination*

COUMARIN IN 50 CC.	VANILLIN IN 50 CC.		READING FILTER NO. 49 AFTER—	
			2 HRS.	24 HRS.
mg.	*mg.*	*ratio, 1 to—*		
0.3	0.0		72.9	80.4
0.3	0.5	1.7	71.5	80.2
0.3	1.0	3.3	71.1	80.9
0.3	1.5	5.0	70.3	81.7
0.3	2.5	8.3	70.1	79.8
0.3	4.0	13.3	68.8	80.8
0.3	5.0	16.6	71.7	82.1
0.3	7.5	25.0	71.1	83.7
0.3	10.0	33.3	71.5	88.0*
			Av. 71.0	81.2

* Omitted from the average.

TABLE 3.—*Effect of varying proportions of heliotropin on coumarin determination*

COUMARIN IN 50 CC.	HELIOTROPIN IN 50 CC.		READING FILTER NO. 49 AFTER—	
			2 HRS.	24 HRS.
mg.	*mg.*	*ratio, 1 to—*		
0.3	0.0		75.9	80.2
0.3	0.0		76.2	80.7
0.3	5.0	17	76.0	80.2
0.3	10.0	34	76.4	80.4
0.3	15.0	50	76.6	80.6
0.3	20.0	67	77.5	80.3

Experiments were then conducted to determine the applicability of a clarification procedure such as is used in the Folin-Denis colorimetric method for vanillin. Since it was evident that the addition of sodium carbonate as a reagent in the colorimetric method for coumarin would cause a precipitate with any residual lead acetate, it was decided to remove the lead with sodium oxalate before applying the color reaction. In order to increase the range of reading in the spectrophotometer it was decided also to increase the quantities of reagent. The method given below was tried out.

COUMARIN IN IMITATION VANILLA

REAGENTS

Standard coumarin solution.—Dissolve 0.1000 gram of pure coumarin in 10 cc. of alcohol and dilute to 100 cc. with water. To prepare a solution of coumarin containing 0.1 mg. per 1 cc., dilute 10 cc. of the standard solution to 100 cc. with water.

Lead acetate solution.—Dissolve 50 grams of neutral Pb acetate and 50 grams of basic Pb acetate in hot water, dilute to 1 liter, cool, and filter.

Sodium oxalate.—Anhydrous.

Sodium carbonate solution.—Dissolve 5 grams of anhydrous Na_2CO_3 in water and dilute to 500 cc.

Solution A: Dissolve 0.7 gram of *p*-nitraniline in 9 cc. of HCl and dilute to 100 cc. with water.

Solution B: Dissolve 5 grams $NaNO_2$ in water and dilute to 100 cc.

Diazonium solution.—Chill a 100 cc. flask and Solutions A and B to about 3° C. in a refrigerator or cold room or in chopped ice. Pipet 5 cc. of each solution into the flask, mix, and let stand in refrigerator 5 minutes. Add 10 cc. more of Solution B, return to the refrigerator for 5 minutes, then fill the flask to the mark with ice-cold water. The solution is ready for use in 15 minutes but must be discarded after 24 hours.

PREPARATION OF GRAPH

Place 1, 3, and 5 cc. portions of coumarin solution (1 cc. = 0.1 mg.) in as many 100 cc. volumetric flasks and add enough water to bring the volume to 20 cc.; add 10 cc. of Na_2CO_3 solution and heat on a water bath at 85° for 15 minutes or in a boiling water bath for 5 minutes. Allow the solutions to cool gradually; when they have reached room temperature, add 10 cc. of diazonium solution to each, fill to the mark with water, and mix. Let stand 1 5–2.0 hours and read in a spectrophotometer, using a No. 49 filter and a ½ inch cell. Plot the results on coordinate paper so that the quantity of coumarin can be read in terms of grams per 100 cc. of the original sample.

DETERMINATION

Pipet 5 cc. of imitation vanilla into a 100 cc. volumetric flask, and add 75 cc. of tap water and 5 cc. of Pb acetate solution. Fill to the mark with tap water. Mix, and filter through a folded filter. To the filtrate add 0.2 gram of anhydrous Na oxalate and dissolve in the filtrate by rotating the container. After the reagent has dissolved completely, rotate again for a few seconds, let stand at least 5 minutes, and filter through a 11 cm. S & S filter 589.

Transfer 5 cc. of the filtrate to a 100 cc. volumetric flask and treat in the same manner as were the standards used in preparing the graph. The final solution has been subjected to a dilution of 400 times if the quantities recommended were used.

The above procedure was applied to a set of imitation vanillas of known composition. The results given in Table 4 were obtained.

The data in Table 4 show that results of reasonable accuracy can be obtained when the colorimetric method for coumarin is applied to imitation vanilla clarified with lead acetate and that the distillation with steam under reduced pressure, as recommended by Duncan and Dustman, may be dispensed with when quick determination of coumarin is desired.

When the method was applied to several vanilla extracts purchased on the open market, the readings obtained showed substantial amounts of coumarin when there was no indication of its presence from the taste or odor of the extract. Further work should be done to establish the composition of the substance in the vanilla that gives a reaction similar to that of coumarin under these circumstances. The data given in this paper clearly show that the color obtained on vanilla extract is not due to vanillin as was reported by Duncan and Dustman. The proportions of

TABLE 4.—*Coumarin in imitation vanilla*

VANILLIN	COUMARIN	VANILLA EXTRACT	CARAMEL TO COLOR	COUMARIN FOUND
g./100 cc.	*g./100 cc.*	*cc./1000 cc.*		*g./100 cc.*
				0.147
0.60	0.151	50	+	0.152
				0.144
				0.058
0.65	0.050	40	+	0.064
				0.06
				0.174
0.70	0.175	60	+	0.164
				0.180
				0.196
0.72	0.200	30	+	0.204
				0.104
0.50	0.100	90	+	0.088
				0.110

coumarin indicated by this method as being present in the true vanilla samples were 0.057, 0.035, 0.09, 0.03, 0.038, and 0.04 gram per 100 cc. It will be remembered, of course, that the official gravimetric method for coumarin frequently yields as much as 0.04 gram per 100 cc. of extract (8) that does not respond to qualitative tests for coumarin nor does the residue have an odor in any way similar to coumarin.

SUMMARY

The condensation of coumarin with diazo-*p*-nitraniline has been shown to be applicable to imitation vanilla when clarified with lead acetate solution, thus forming the basis of a quick method for the determination of coumarin in imitation vanilla.

True vanilla extracts have been shown to contain a substance, not vanillin, that yields color with the reagent, so that in the case of true vanilla the test should be applied to a distillate, as recommended by Duncan and Dustman, before it may be concluded that this ingredient has been added to the vanilla.

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A COMPARISON OF THE OFFICIAL AND MACINTIRE-SHAW-HARDIN METHODS FOR DETERMINING AVAILABLE PHOSPHORIC ACID

By J. RICHARD ADAMS (Bureau of Chemistry and Soils, Washington, D. C.)

At the 1937 meeting of the Association of Official Agricultural Chemists a paper was presented by MacIntire, Shaw, and Hardin[1] on a direct method for the determination of available P_2O_5. The method is claimed by the authors[2] to be a simple, rapid, and economical direct analytical procedure applicable to all types of phosphatic fertilizers. It consists in leaching a weighed sample of the fertilizer with 100 cc. of an ammonium nitrate-ammonium citrate solution of pH 4.2. The leached residue is then steam digested in another 100 cc. portion of this special citrated ammonium nitrate solvent for 30 minutes. The leachate and digestate are combined, made up to volume, and filtered. The P_2O_5 content of the filtrate is determined by the official method. This value is taken as a measure of the available P_2O_5 of the sample. The MacIntire-Shaw-Hardin method is thus a direct one in that the available P_2O_5 is determined as such rather than by the difference between the total and citrate-insoluble P_2O_5 as in the official method.

In fertilizer laboratories records are kept of both the total and citrate-insoluble forms of P_2O_5, and the available P_2O_5 obtained by the official method entails no additional determination. In over 80 per cent of the control laboratories, however, only the available P_2O_5 in a sample is required and in these laboratories the MacIntire-Shaw-Hardin method would offer an advantage over the official method in that it involves only one determination.

Because of the possibilities of this proposed method, it was suggested that a comparative study should be made of the official and MacIntire-Shaw-Hardin methods for determining available P_2O_5. In compliance with this suggestion and under the direction of the Associate Referee on Phosphoric Acid, the work presented here was carried out in order to determine how close an agreement exists between these two methods when used to determine available P_2O_5 in various phosphatic materials.

The steam digestion apparatus used is somewhat different from the one described by MacIntire and coworkers. The modified apparatus is shown in Figure 1. Steam, generated in a one-half horse power boiler, is regulated by means of needle valves in the manifold and is injected into the boiling solution through a tube having a spiral outlet. This ensures a swirling motion of the steam in the solution and causes complete agitation. A pressure release tube opening into a trap on the back of the

[1] *This Journal*, 21, 113 (1938).
[2] *Ind. Eng. Chem. Anal. Ed.*, 10, 143 (1938).

apparatus prevents any mechanical loss of the solution. The solution is maintained at a constant volume of 100 cc. by proper regulation of the steam and the burner temperature.
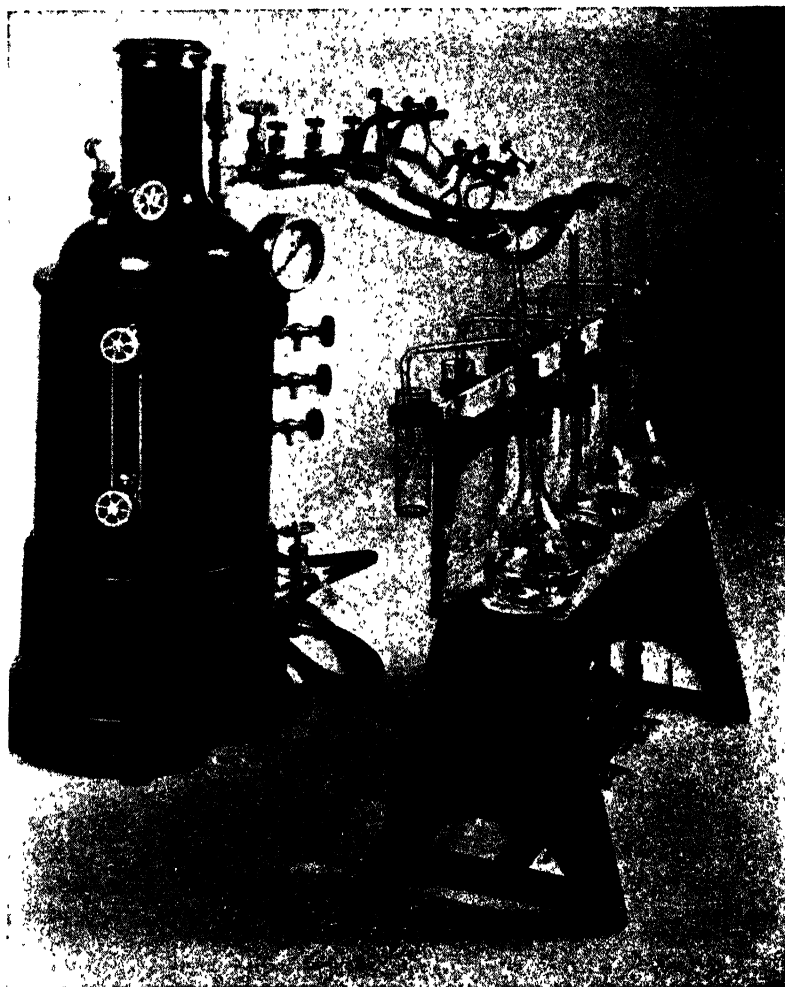

FIG. 1.—APPARATUS FOR DETERMINING AVAILABLE P₂O₅ BY THE MACINTIRE-SHAW-HARDIN METHOD.

It was found necessary to exercise some care in the use of the citrated ammonium nitrate solution because of its tendency to mold. This mold formation increases the acidity of the solution and consequently gives high availability values. The mold forms only when the solution is allowed to stand for several weeks. Any possibility of trouble from this source can be avoided by preparing only enough solution for the work at hand.

The samples analyzed cover a wide range of availability and include

monopotassium phosphate, di- and tricalcium phosphates, calcium hydroxyphosphate, calcined phosphate, ammoniated superphosphate, steamed bone meal, basic slag, raw rock phosphates, and mixtures containing one or more of these phosphatic components in different combinations. The samples, in all cases, were ground to pass an 80-mesh sieve, and the analyses were made in accordance with the published procedures. The results of both methods of analysis are given in the table.

The tabulated figures are the average of closely agreeing duplicates obtained on each of two samples, or four determinations in all. Fully as good agreement was obtained between duplicates by the MacIntire-Shaw-

Available P_2O_5 in various phosphatic materials as determined by the A.O.A.C. and MacIntire-Shaw-Hardin methods

SAMPLE	A.O.A.C. METHOD				MACINTIRE-SHAW-HARDIN METHOD		
	TOTAL P_2O_5	CITRATE-INSOLUBLE P_2O_5	AVAILABLE P_2O_5	AVAILABLE P_2O_5, % OF TOTAL	WT. OF SAMPLE	AVAILABLE P_2O_5	AVAILABLE P_2O_5, % OF TOTAL
	per cent	*per cent*	*per cent*		*grams*	*per cent*	
KH$_2$PO$_4$	52.13	0.00	52.13	100.00	0.5	52.43	100.58
Ca$_3$(PO$_4$)$_2$	35.69	21.95	13.74	38.50	0.5	27.06	75.82
CaHPO$_4$	48.53	3.66	44 87	92.46	0 5	46.26	95 32
CaHPO$_4$+CaCO$_3$(1:1)	26.31	5.36	20.95	79.63	1.0	14.74	56.02
CaHPO$_4$+CaSO$_4$(1:1)	26.69	0.18	26.51	99.33	1.0	23.58	88.34
Calcium hydroxyphosphate*	41.83	31.46	10 37	24.79	0.5	41.04	98 11
Calcium hydroxyphosphate*					1.0	36 05	86 18
Calcium hydroxyphosphate*,b	43.57	26.80	16.77	38.49	0.5	30 12	69 13
Calcined phosphate	37.05	21.32	15 73	42.46	1.0	21.99	59 35
Steamed bone meal	34.42	18.52	15.90	46.19	1.0	32.59	94.68
Basic slag	11.28	9.86	1.42	12.59	1.0	3.15	27.93
Florida pebble phosphate*	35.25	32.72	2.53	7.17	1.0	3.76	10.58
Florida pebble phosphate	30.88	27.45	3.43	11.09	1.0	4.26	13.61
Tenn. brown rock phosphate^d	32.82	30.32	2.50	7.71	1.0	3.10	9 38
Tenn. brown rock phosphate	33.77	31.19	2.58	7.05	1.0	3.12	9.24
Ammoniated superphosphate*	19.76	1.33	18.43	93.27	1.0	19.74	99.90
Ammoniated superphosphate^f	18.40	4.13	14.27	77.55	1.0	18.20	98.91
6-8-4 mixture contg. raw rock	21.88	13.77	8.11	37.07	1.0	10.88	49.73
5-10-5 mixture contg. C.S.M.	11.29	0.22	11.07	98.05	1.0	11.20	99.20
4-8-4 mixture	8.16	0.13	8.03	98.41	1.0	8.01	98.16
4-16-4 mixture	15.43	1.01	14.42	93.45	1.0	15.56	100.84
1938 A.O.A.C. sample No. 1	17.57	3.22	14.35	81.67	1.0	17.17	97.72
1938 A.O.A.C. sample No. 2	15.69	4.69	11.00	70.11	1.0	14.38	91.65
1938 A.O.A.C. sample No. 3	40.19	2.45	37.74	93.90	1.0	40.50	100.77
1938 A.O.A.C. sample No. 4	40.17	3.05	37.12	92.41	1.0	39.77	99.00

a P_2O_5/CaO =0.788; theoretical =0.760.
b Heated in an atmosphere of steam for 30 minutes at 1400° C.
c Bureau of Standards Standard Sample No. 120.
d Bureau of Standards Standard Sample No. 56a.
e NH$_3$ =4.3%.
f NH$_3$ =6.8%.

Hardin method as by the official method. In 50 per cent of the samples analyzed very good agreement was found to exist between the results obtained by the two methods. The MacIntire-Shaw-Hardin method gave high results for 42 per cent of the samples. These high values were particularly marked in the case of tricalcium phosphate and calcium hydroxyphosphate. The trend towards the higher availability values tends to fall in line with the published results of vegetative tests[3] and adds greatly to the promise of the MacIntire-Shaw-Hardin method. However, as both of the methods under discussion are of an arbitrary nature, further vegetative tests would seem to be advisable before their relative accuracy can be finally estimated.

The Associate Referee on Phosphoric Acid has made a recommendation[4] that further consideration be given to the MacIntire-Shaw-Hardin method for the determination of available P_2O_5.

EFFECT OF FLUORINE IN THE DETERMINATION OF CITRATE-INSOLUBLE PHOSPHORIC ACID BY THE OFFICIAL METHOD

By L. F. RADER, JR., and WILLIAM H. ROSS (Fertilizer Research Division, Bureau of Chemistry and Soils, Washington, D. C.)

At the last meeting of this Association a report was presented by Ross, Rader and Beeson[1] on the "Citrate-insoluble Phosphoric Acid in Ammoniated Mixtures Containing Dolomite." In the study that was made of this subject it was found that:

(1) The citrate-insoluble P_2O_5 in a fluorine-free ammoniated superphosphate remained unchanged when stored with or without dolomite at 30° C. for 180 days.

(2) Storage of a fluorine-free ammoniated superphosphate at 75° C. for the same length of time caused a slight increase in citrate-insoluble P_2O_5 in the absence of dolomite and a marked increase when dolomite was present.

(3) The presence of fluorine as calcium fluoride in an ammoniated superphosphate caused a slight increase in citrate-insoluble P_2O_5 when stored at 30° C. for 180 days in the absence of dolomite and a marked increase in the presence of dolomite.

(4) An increase in the storage temperature of a fluorine-containing ammoniated superphosphate or ammoniated superphosphate-dolomite mixture from 30 to 75° C. reduced still further the availability of the P_2O_5 in the mixture.
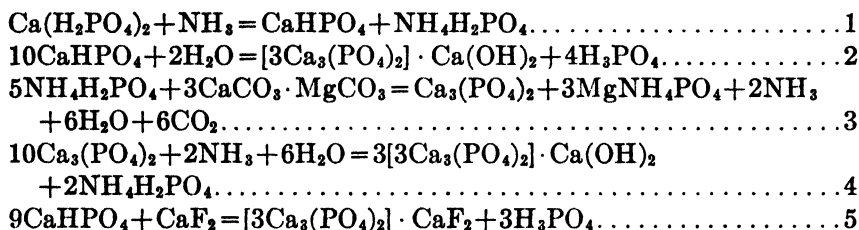
These results may be explained if it is assumed that the dicalcium phosphate initially formed in the ammoniation of superphosphate (Equation 1) undergoes hydrolysis in storage at temperatures above normal to form calcium hydroxyphosphate as represented in Equation 2, and that the presence of dolomite increases the extent to which calcium hydroxy-

[3] Ross, Jacob, and Beeson, *This Journal*, 15, 227 (1932).
[4] Ross and Adams, *This Journal*, 22, 254 (1939).
[1] *This Journal*, 21, 258 (1938).

phosphate is formed by reacting at temperatures above normal with the monoammonium phosphate formed by ammoniation (Equation 1) as indicated in Equations 3 and 4. In the presence of fluorine, the reactions apparently proceed to the formation of fluorapatite as suggested by MacIntire and his coworkers.[2] If this reaction proceeds as indicated in Equation 5, any increase in citrate-insoluble P_2O_5 should be accompanied by a corresponding increase in water-soluble P_2O_5.

$$Ca(H_2PO_4)_2 + NH_3 = CaHPO_4 + NH_4H_2PO_4 \dots\dots\dots\dots\dots\dots\dots 1$$

$$10CaHPO_4 + 2H_2O = [3Ca_3(PO_4)_2] \cdot Ca(OH)_2 + 4H_3PO_4 \dots\dots\dots\dots 2$$

$$5NH_4H_2PO_4 + 3CaCO_3 \cdot MgCO_3 = Ca_3(PO_4)_2 + 3MgNH_4PO_4 + 2NH_3$$
$$+ 6H_2O + 6CO_2 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots 3$$

$$10Ca_3(PO_4)_2 + 2NH_3 + 6H_2O = 3[3Ca_3(PO_4)_2] \cdot Ca(OH)_2$$
$$+ 2NH_4H_2PO_4 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots 4$$

$$9CaHPO_4 + CaF_2 = [3Ca_3(PO_4)_2] \cdot CaF_2 + 3H_3PO_4 \dots\dots\dots\dots\dots 5$$

That an increase in water-soluble P_2O_5 does take place in mixtures of an ammoniated superphosphate and calcium fluoride was shown by the results given in last year's report.[1] If the reactions taking place when dolomite is added to a mixture of this kind proceed as represented in Equations 3, 4 and 5, then there should be at first a decrease and later an increase in water-soluble P_2O_5. This was found to be true by chemical analysis.

EXPERIMENTAL

In continuing the work as recommended at the last meeting of the Association,[1] the writers made further tests on the effect of storage at temperatures above normal on the citrate-insoluble P_2O_5 in commercial ammoniated superphosphates. All tests were made with samples prepared by ammoniation of the same Tennessee superphosphate. The results obtained (Table 1) agree with those previously reported[1] in showing that the citrate-insoluble P_2O_5 in a commercial ammoniated superphosphate increases slowly at 30° C. and more rapidly at higher temperatures. Tests made with an ammoniated superphosphate of varying particle size (Samples 181–211) indicate that the size of the particle has little or no effect on the reversion of the P_2O_5 in storage.

In a second series of tests, samples of ammoniated superphosphates were stored at a temperature below normal (5° C.) as well as at the higher temperatures. Two of the samples used in these experiments were commercial ammoniated superphosphates and the third was a synthetic product prepared by ammoniating a mixture of C.P. monocalcium phosphate and calcium fluoride.* The results given in Table 2 show that ammoniated superphosphate mixtures of the ordinary type can be stored at 5° for 180 days with no significant loss of available P_2O_5, but that

[2] Ind. Eng. Chem., 10, 143 (1938); *This Journal,* 21, 113 (1938).
* The "C.P." calcium fluoride used in this test was found to contain a small amount of a soluble fluoride. It was replaced in subsequent tests with a higher grade calcium fluoride.

TABLE 1.—*Effect of storage on the citrate-insoluble P_2O_5 in commercial ammoniated superphosphates*

SAMPLE NO.	PARTICLE SIZE	NH₃	PHOSPHORIC ACID (P_2O_5)		INCREASE IN CITRATE-INSOLUBLE P_2O_5 AFTER STORAGE FOR 6 MONTHS AT		
			TOTAL	CITRATE-INSOLUBLE[a]	30°	45°	75°
	mesh			*per cent*			
223	< 8	2.78	21.04	0.53	0.92	1.44	2.85
181[b]	>10	1.98	21.18	0.50	0.59	0.88	2.22
191[b]	10–20	2.20	21.40	0.46	0.46	0.79	1.34
201[b]	20–40	2.52	22.07	0.59	0.56	1.04	2.14
211[b]	<40	2.46	20.43	0.60	0.75	0.65	1.65
221	< 8	0.93	21.32	0.43	0.14	—	1.37
222	< 8	1.49	21.54	0.45	0.30	—	1.65
241	<20	2.24	20.08	0.43	0.12	0.44	1.47
231	<40	2.25	21.32	0.51	0.59	0.49	1.59

[a] All samples ground to pass a 40-mesh screen before analysis.
[b] Prepared by screening Sample 223.

TABLE 2.—*Per cent citrate-insoluble P_2O_5 in ammoniated superphosphates stored at temperatures above and below normal*
(Ammonia content = 3%[a])

SAMPLE NO.	SOURCE OF PHOSPHATE	TOTAL P_2O_5	INITIAL CITRATE-INSOLUBLE P_2O_5	INCREASE IN CITRATE-INSOLUBLE P_2O_5 AFTER STORAGE AT					45° FOR 180 DAYS
				5° FOR			30° FOR		
				30 DAYS	60 DAYS	180 DAYS	15 DAYS	180 DAYS	
161	Florida pebble rock	20.66	0.64	0.00	0.00	0.00	—	2.16	3.91
171	Tennessee brown rock	20.16	0.54	0.00	0.00	0.04	—	1.71	2.51
471	$Ca(H_2PO_4)_2$ $+CaF_2$[b]	52.25	0.20	0.18	0.28	1.18	3.50	—	—

[a] On basis of superphosphate containing 20% P_2O_5
[b] Fluorine in ammoniated product = 1.5%.

some increase in citrate-insoluble P_2O_5 occurs at this temperature in the synthetic ammoniated product. This is in agreement with the previous observation[1] that in ammoniated mixtures of pure materials containing fluorine the rate of the reversion of P_2O_5 is much greater than it is in the corresponding commercial products.

REVERSION OF P_2O_5 IN THE PROCESS OF ANALYSIS

If the loss of available P_2O_5 that occurs in ammoniated superphosphate mixtures during storage is due to the reaction represented in Equation 5, then the rate of the reaction should be increased by any treatment, such as washing, that would remove the free phosphoric acid formed. It has

also been observed by Ross and Jacob[3] that tricalcium phosphate is changed into calcium hydroxyphosphate by digestion in neutral ammonium citrate solution. It seemed possible, therefore, that a portion of the citrate-insoluble P_2O_5 found in ammoniated mixtures might be due to a reversion of the phosphoric acid in the process of analysis.

In this study of the problem, samples were prepared that contained varying combinations of the phosphatic compounds commonly occurring in ammoniated fertilizer mixtures. The materials and the proportion of each used in the preparation of the samples were as follows:

MATERIAL	PERCENTAGE COMPONENTS OF SAMPLE				
	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5
Ammonia	7	—	7	—	—
Monoammonium phosphate	—	—	—	5	—
Monocalcium phosphate monohydrate	88	—	57	—	—
Dicalcium phosphate, dihydrate	—	95	31	55	—
Tricalcium phosphate, anhydrous	—	—	—	—	95
Tricalcium phosphate, precipitated	—	—	—	25	—
Ammonium sulfate	—	—	—	10	—
Quartz flour	5	5	5	5	5
Total	100	100	100	100	100

All materials were ground to pass a 100-mesh sieve before being mixed. The composition of the samples is given in Table 3.

TABLE 3.—*Analysis of standard phosphate samples*

SAMPLE NO.	NH₃	CaO	TOTAL P₂O₅
		per cent	
1	7.31	18.59	51.33
2	—	31.00	39.13
3	6.90	25.30	47.27
4	3.31	35.46	40.17
5	—	51.33	43.02

FRESHLY-PREPARED MIXTURES

Samples 1 to 5 were analyzed for citrate-insoluble P_2O_5 as prepared and again when the quartz flour was replaced in part with a sufficient quantity of a fluorine compound to give a dry mixture containing 1.5 per cent of fluorine. The fluorine compound was mixed with each of the samples and the resulting mixture was then analyzed immediately to insure against any reversion of the P_2O_5 other than that which occurred during the process of analysis.

[3] *This Journal*, **14**, 182 (1931).

The results obtained, given in the first column of figures in Table 4, show (1) that the presence of calcium fluoride causes little or no increase in citrate-insoluble P_2O_5 during the process of analysis of freshly-prepared mixtures over that found when fluorine is absent; and (2) that a marked reversion of P_2O_5 occurs during the process of analysis of freshly-prepared mixtures containing sodium fluoride or other water-soluble fluorine compound.

TABLE 4.—*Variation in citrate-insoluble P_2O_5 during washing and digestion of freshly-prepared phosphatic mixtures containing water-soluble or water-insoluble fluorides*

MIXTURE	PER CENT CITRATE-INSOLUBLE P_2O_5					
	TIME OF WASHING[a]			TIME OF DIGESTION[b]		
	1 HR.	4 HRS.	24 HRS.	1 HR.	4 HRS.	24 HRS.
Sample 1 with quartz	0.00	0.00	0.00	0.00	0.00	0.00
Sample 1 with CaF₂	0.00	0.00	0.00	0.00	0.00	0.00
Sample 1 with NaF	1.03	2.63	3.95	1.03	1.10	3.27
Sample 2 with quartz	0.00	0.00	0.00	0.00	0.00	0.00
Sample 2 with CaF₂	0.00	0.00	0.00	0.00	0.00	0.00
Sample 2 with NaF	0.30	0.95	5.15	0.30	0.40	5.90
Sample 3 with quartz	0.08	0.06	0.07	0.08	0.07	0.11
Sample 3 with CaF₂	0.08	0.08	0.15	0.08	0.08	1.43
Sample 3 with NaF	4.57	5.05	7.05	4.57	4.46	7.09
Sample 3 with NH₄F	5.20	4.95	7.12	5.20	4.20	8.21
Sample 3 with Na₂SiF₆	4.05	4.08	5.60	4.05	4.95	6.65
Sample 3 with CaSiF₆ ·2H₂O	6.43	6.95	9.03	6.43	5.68	7.73
Sample 3 with CaCl₂	0.00	0.00	0.00	0.00	0.00	0.40
Sample 4 with quartz	6.30	6.26	6.59	6.30	6.13	7.73
Sample 4 with CaF₂	5.95	6.20	6.68	5.95	6.18	9.15
Sample 4 with NaF	9.18	11.60	15.50	9.18	9.76	15.18
Sample 4 with CaSO₄ ·2H₂O	5.99	6.40	6.47	5.99	6.24	7.48
Sample 5 with quartz	24.75	25.00	24.70	24.75	21.25	16.25
Sample 5 with CaF₂	25.26	25.44	25.77	25.26	21.51	17.00
Sample 5 with NaF	26.57	26.19	26.72	26.57	23.28	17.31

[a] Time of digestion in citrate solution, 1 hour.
[b] Time of washing, 1 hour.

The effect of varying the time of washing and of the citrate digestion of the washed residues on the reversion of the P_2O_5 in the samples was determined by varying the period of either treatment from 1 to 24 hours while the other treatment remained constant. The quantity of wash water used in each determination was limited to 250 cc. The results obtained (Table 4) indicate that fluorine-free mixtures undergo little or no increase in citrate-insoluble P_2O_5, either with prolonged washing of the sample or when the washed residues are digested in citrate solution for a period of 4 hours or less. Sample 4, which contained precipitated

tricalcium phosphate, showed some increase in citrate-insoluble P_2O_5 when the washed residue was digested for 24 hours, whereas the citrate-insoluble P_2O_5 in Sample 5, which contained anhydrous tricalcium phosphate as the only phosphatic component, decreased on prolonged digestion of the washed residue. Ross, Jacob, and Beeson[4] found that precipitated or hydrated tricalcium phosphate is more soluble in citrate solution than the anhydrous or ignited product. The opposite results obtained with Samples 4 and 5 indicate that the digestion of Sample 5 in citrate solution results not only in a partial hydrolysis of the anhydrous tricalcium phosphate but also in its partial hydration to the more soluble hydrated form.

Table 4 further shows that prolonged washing of the sample, or a 1–4 hour digestion in citrate solution subsequent to washing, causes little or no increase in citrate-insoluble P_2O_5 when calcium fluoride is included in the mixture, and that a marked increase in citrate-insoluble P_2O_5 takes place under the same conditions when the calcium fluoride is replaced by a more soluble fluoride or fluosilicate. No appreciable increase in citrate-insoluble P_2O_5 takes place, however, when the fluorine compound is replaced by a calcium salt such as the sulfate or chloride.

The results obtained when Samples 1 and 3 alone and in mixture with calcium fluoride were digested in citrate solution without previous washing are given in Table 5. A comparison of these results with those in Table 4 shows that failure to remove soluble phosphates by washing increases the rate at which samples containing fluorine undergo reversion during digestion in citrate solution.

TABLE 5.—*Variation in citrate-insoluble P_2O_5 with time of digestion without prior washing with water*

| | PER CENT CITRATE-INSOLUBLE P_2O_5 | | |
| | TIME OF DIGESTION | | |
MIXTURE	1 HR.	4 HRS.	7 DAYS
Sample 1 with quartz	0.00	0.00	0.00
Sample 1 with CaF_2	0.25	0.17	10.02
Sample 3 with quartz	0.08	0.17	0.44
Sample 3 with CaF_2	0.85	0.89	14.90

STORED MIXTURES

It was shown by Ross, Rader, and Beeson[1] that ammoniated mixtures containing calcium fluoride undergo slight reversion in storage at ordinary temperatures and a more rapid reversion at higher temperatures. Tests were accordingly undertaken to determine whether the reversion that takes place during storage is accompanied by a further reversion

⁴ *This Journal*, 15, 227 (1932).

during the process of analysis by the present official method. In this series of experiments Sample 3, in mixture with calcium fluoride and again with sodium fluoride, was stored with a moisture content of 7 per cent for 7 and for 28 days at 20°–25° C. and for 7 and 21 days at 100° C. Mixtures of Sample 2 with calcium fluoride and with sodium fluoride were also stored for 7 and for 21 days at 100° C.

TABLE 6.—*Effect of storage on citrate-insoluble P_2O_5 in phosphatic mixtures containing water-soluble or water-insoluble fluorides*

MIXTURE	WATER-SOLUBLE		CITRATE-INSOLUBLE	
	P_2O_5	F	P_2O_5	F
	per cent			
Fresh mix, analyzed at once				
Sample 2 with CaF$_2$	1.45	0.01	0.00	1.18
Sample 2 with NaF	9.98	0.43	0.30	0.62
Sample 3 with CaF$_2$	19.33	0.01	0.08	1.22
Sample 3 with NaF	27.33	0.48	4.57	0.33
Stored 7 days at 20°–25° C.				
Sample 3 with CaF$_2$	19.30	0.01	0.00	1.15
Sample 3 with NaF	27.34	0.02	13.05	0.68
Stored 28 days at 20°–25° C.				
Sample 3 with CaF$_2$	19.56	0.01	0.10	1.16
Sample 3 with NaF	27.61	0.01	14.02	0.74
Stored 7 days at 100° C.				
Sample 2 with CaF$_2$	7.74	0.02	1.19	1.40
Sample 2 with NaF	15.61	0.02	17.20	1.48
Sample 3 with CaF$_2$	25.65	0.01	18.60	1.22
Sample 3 with NaF	27.30	0.02	17.14	1.00
Stored 21 days at 100° C.				
Sample 2 with CaF$_2$	1.52	—	5.27	0.87
Sample 2 with NaF	12.46	—	17.67	1.27
Sample 3 with CaF$_2$	25.63	—	19.40	1.15
Sample 3 with NaF	28.05	—	16.83	0.83

The results obtained (Table 6) show that the citrate-insoluble P_2O_5 found in the calcium fluoride mixtures stored at normal temperatures was little, if any, greater than in the freshly-prepared mixtures. When stored at 100° C. the calcium fluoride as well as the sodium fluoride mixtures undergo a marked increase in citrate-insoluble P_2O_5 over the corresponding freshly-prepared mixtures. The data in Table 6 further show that in the sodium fluoride mixtures the water-soluble fluorine decreases while the citrate-insoluble fluorine increases with storage. This affords further evidence of fluorapatite formation in ammoniated mixtures or mixtures containing di- or tricalcium phosphates when fluorine is present.

The citrate-insoluble P_2O_5 in the stored mixtures listed in Table 7 was determined by washing and by digesting the washed residues for 1 hour each as directed in the present official method. The results obtained when each treatment was increased to 6 hours and to 24 hours while the other treatment remained at 1 hour are also shown in Table 7. The data given in the table are in general agreement with the results obtained in the

TABLE 7.—*Variation in citrate-insoluble P_2O_5 during washing and digestion of stored phosphatic mixtures containing water-soluble or water-insoluble fluorides*

MIXTURE	PER CENT CITRATE-INSOLUBLE P_2O_5					
	TIME OF WASHING[a]			TIME OF DIGESTION[b]		
	1 HR.	6 HRS.	24 HRS.	1 HR.	6 HRS.	24 HRS.
Stored 7 days at 20°–25° C.						
Sample 1 with NaF	10.76	10.87	11.53	10.76	10.62	10.29
Sample 2 with NaF	12.90	15.57	16.28	12.90	12.67	12.82
Stored 28 days at 20°–25° C.						
Sample 3 with CaF_2	0.10	0.10	0.20	0.10	0.10	1.50
Sample 3 with NaF	14.02	14.16	14.54	14.02	13.56	14.30
Stored 7 days at 100° C.						
Sample 1 with CaF_2	13.42	13.62	14.00	13.42	12.96	11.91
Sample 1 with NaF	13.01	14.64	15.79	13.01	12.00	12.35
Sample 2 with CaF_2	1.19	1.24	1.38	1.19	1.48	1.46
Sample 2 with NaF	17.20	17.22	17.69	16.72	16.83	16.97
Stored 21 days at 100° C.						
Sample 3 with CaF_2	19.40	19.52	19.48	19.40	18.83	18.23
Sample 3 with NaF	16.83	16.67	16.57	16.83	16.27	15.63

[a] Time of digestion in citate solution, 1 hour.
[b] Time of washing, 1 hour.

collaborative study that was made of the extent to which the citrate-insoluble P_2O_5 in phosphatic mixtures varied with the time interval between the water extraction and citrate digestion.[5] This study showed that in ammoniated mixtures containing fluorine there was a slight but more or less constant increase in citrate-insoluble P_2O_5 with the time interval between washing and citrate digestion. The results given in Table 7 indicate that the citrate-insoluble P_2O_5 in stored ammoniated mixtures containing fluorine also undergo a more or less constant increase with the time of washing and with the time of digestion in citrate solution. This rate of increase in the case of the stored samples is such as to indicate a small but insignificant increase during the time (1 hour) specified in the official method for washing the sample and for making the citrate digestion.

[5] Ross and Adams, *This Journal*, 22, 254 (1939).

SUMMARY AND CONCLUSIONS

1. Neither freshly-prepared nor stored fluorine-free phosphate mixtures undergo any significant increase in citrate-insoluble P_2O_5 during the process of analysis when each of the different steps in the procedure is completed within one hour.

2. The presence of calcium fluoride causes little or no increase in citrate-insoluble P_2O_5 during the process of analysis of freshly-prepared ammoniated mixtures or mixtures containing di- or tricalcium phosphate over that found when fluorine is absent.

3. A marked reversion of P_2O_5 occurs during the process of analysis of freshly-prepared ammoniated mixtures or mixtures containing di- or tricalcium phosphate in the presence of sodium fluoride or other water-soluble fluorine compound.

4. In the presence of calcium fluoride, ammoniated mixtures or mixtures containing di- or tricalcium phosphate undergo a very slow reversion in storage at ordinary temperatures. Increasing the temperature of storage or replacing the calcium fluoride with sodium fluoride increases the rate of reversion.

5. In the presence of a fluorine compound, stored ammoniated mixtures may undergo a small increase in citrate-insoluble P_2O_5 during the process of analysis, but the increase is insignificant if each of the different steps in the procedure is completed within a period of one hour.

DETERMINATION OF ROTENONE IN DERRIS AND CUBE POWDERS

USE OF DECOLORIZING CARBON IN THE CHLOROFORM EXTRACTION METHOD

By J. J. T. GRAHAM (Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

In the determination of rotenone in derris and cube powders, several difference solvents and methods of extraction have been proposed, but in all the recent methods carbon tetrachloride has been used exclusively as a crystallizing medium.

It was observed by Jones and Graham[1] that in general it is easier to get complete extraction of the rotenone from cube than from derris, but that the rotenone crystallizes more slowly from the cube extracts than from derris extracts of comparable rotenone content; furthermore, the crystalline precipitates from the cube extracts are usually more highly colored and retain the resin contaminations more tenaciously.

It has also been recognized by a number of workers[2,3,4,5] that the derris

[1] *Ind. Eng. Chem. Anal. Ed.,* **10,** 19 (1938).
[2] Cahn and Boam, *J. Soc. Chem. Ind.,* **54,** 37T (1935).
[3] Jones, H. A., *Ind. Eng. Chem. Anal. Ed.,* **9,** 206 (1937).
[4] Seaber, W. M., *J. Soc. Chem. Ind.,* **56,** 168T (1937).
[5] Worsley, R. R. Le G., *Ibid.,* **55,** 349T (1936).

and cube resins interfere with the crystallization of the rotenone from the carbon tetrachloride solution. However, the work of Jones[3] indicates that this can be overcome by the addition of sufficient pure rotenone in the crystallization medium to bring the concentration up to a certain minimum quantity, provided the crystallization is allowed to proceed for a sufficient period of time.

After a study of the more promising methods, Jones and Graham[1,6] recommended a procedure that is essentially a combination of the chloroform extraction method of Beach[7] and the crystallization method of Jones.[3]

Recently the suggestion was made to the writer* that higher results for rotenone on cube samples could be obtained by the Jones-Graham method by the addition of decolorizing carbon in the extraction flasks. Accordingly a series of cube and derris samples was analyzed for rotenone by the method as published[6] and also with the addition of decolorizing carbon (Norit-A was used in this work). The sample and the carbon were mixed in the flask before the chloroform was added for extraction. The results of these analyses are given in Table 1.

TABLE 1.—*Results for rotenone and total chloroform extract on cube and derris powders*
(30 grams of sample and 300 cc. of chloroform used)

SAMPLE		ROTENONE		PURITY OF THE SOLVATE		CHLOROFORM EXTRACT	
		WITHOUT CARBON	10 g.* CARBON	WITHOUT CARBON	10 g. CARBON	WITHOUT CARBON	10 g. CARBON
		per cent		*per cent*		*per cent*	
3230	Cube	3.8	4.3	77.1	90.9	18.8	15.0
3004	Cube	2.8	3.6	79.2	93.1	16.2	12.9
1938-2	Cube	4.3	5.1	79.6	90.7	22.4	17.7
3596	Cube	3.9	4.5	75.2	88.9	18.7	14.8
NCA	Cube	4.0	4.6	79.5	89.3	—	—
2119	Cube	5.1	5.8	74.7	85.8	19.9	17.0
25095-D	Cube	3.1	3.8	80.3	93.1	15.3	12.0
2711	Cube	1.7	2.2	84.3	97.0	9.1	6.2
3126	Derris	5.6	5.5	86.5	89.8	16.1	14.1
3002	Derris	2.0	2.1	82.7	88.0	12.5	10.2
3006	Derris	3.7	3.8	79.7	84.1	16.2	13.6
1937-1	Derris	4.5	4.6	83.0	92.7	—	—
1938-1	Derris	4.5	4.8	83.5	92.4	16.1	13.7

* Sample 3596 when treated with 15 grams of carbon gave results no different from those obtained by the use of 10 grams of carbon.

In every case the samples of cube gave higher values for rotenone when the carbon was mixed with the powder in the extraction flask, the increase ranging from 0.5 to 0.8 per cent. The differences in the values for rotenone in the derris samples are within the limits of reproducibility of the method.

[6] *This Journal*, 21, 148 (1938).
[7] *Soap*, 12, 109, 111 (1936).
* Credit for this suggestion is due C. A. Greenleaf of the National Canners Association, Washington, D. C.

The quantity of chloroform extract was determined by evaporating 10 cc. of the filtered chloroform solution to dryness and heating at 105° C. to constant weight. The extract in which carbon was used showed a reduction of the dried residue varying from 2.9 to 4.7 per cent in the case of the cube samples and from 2.0 to 2.6 per cent in the derris samples.

Cahn, Phipers, and Boam[8] found that passage of a rotenone-rich extract of derris in benzene twice through a short column of charcoal gave, after removal of the solvent from the percolate, a completely colorless, transparent resin from which rotenone crystallized spontaneously in clusters of radiating needles, but that only a part of the rotenone originally present could be separated, as some was adsorbed on the charcoal.

While determining rotenone in *Mundulea* bark Worsley[5] experienced much trouble with chlorophyl and other coloring compounds that were extracted along with the resins by the ethyl acetate used as a solvent. He found that if the resins were dissolved in alcohol and shaken with charcoal the colors were removed and also a little rotenone, but that no loss of rotenone occurred when the charcoal was first mixed with the powdered bark and then extracted in his special apparatus.

The possibility of loss of rotenone when using decolorizing carbon was recognized by the writer; therefore investigations were conducted to settle this point. It was found that when 1.75 grams of pure rotenone was mixed with 10 grams of carbon and then carried through the extraction, crystallization, and purification procedures about 30 per cent of the rotenone was lost. However, because derris samples analyzed both with and without the use of carbon gave no significant difference in the rotenone values, the indications were that the carbon had no adsorptive effect on the rotenone under the conditions of this analysis, where the other chloroform-soluble constituents were present. To determine this point, one sample of cube and two samples of derris of low rotenone content were selected, and analyses were made with and without the addition of carbon, and with and without the addition of rotenone in the extraction flasks. The rotenone added to the sample in the flask varied from 0.8 to 1.5 grams, and it was mixed with the carbon before the chloroform was added. The results of these analyses are given in Table 2.

In the case of the derris samples the percentages of rotenone recovered in the determinations in which carbon was used agreed with those recovered in the absence of carbon, after corrections for the quantities of rotenone added to the extraction flasks. In the case of the cube sample the recovered rotenone, after correction for that added, checked that obtained when carbon was used and no rotenone was added to the extraction flask, but both results were slightly higher than the result obtained without the use of carbon, which was to be expected. These results indicate that no rotenone was adsorbed by the carbon, otherwise lower results would have been obtained.

[8] *J. Soc. Chem. Ind.*, **57**, 200 (1938).

TABLE 2.—*Recovery of rotenone in presence of carbon and added rotenone in the extraction flasks*

(30 grams of sample and 300 cc. of chloroform used)

SAMPLE		WITHOUT CARBON	10 GRAMS OF CARBON IN EXTRACTION FLASK. ROTENONE ADDED IN EXTRACTION FLASK—			
			NONE	0.8 g.	1.2 g.	1.5 g
		per cent	*per cent*	*per cent**	*per cent**	*per cent**
3354	Derris	0.6	0.8	--	—	0.8
3002	Derris	2.0	2 1	2 1	2.1	—
2711	Cube	1.7	2.2	—	2.2	—

* Percentages of rotenone corrected for the pure rotenone added.

In the extracts obtained with carbon, the rotenone crystallized rapidly and the precipitates were white in contrast to the brown color frequently encountered in the analysis of cube powders. Evidently the carbon removes the portion of the resin that has the greatest retarding effect on the crystallization of the rotenone.

SUMMARY

In the analysis of cube powders by the Jones-Graham method higher percentages of rotenone were obtained, and the rotenone-carbon tetrachloride solvate crystallized more readily and had a purer composition when carbon was used in the extraction flask.

The use of carbon in the extraction flask, in the case of the derris powders tested, caused no significant difference in the results for rotenone.

THE DETERMINATION OF THALLOUS SULFATE IN ANT POISONS

By C. G. DONOVAN (Insecticide Division, U. S. Food and Drug Administration, Washington, D. C.)

The use of thallium as a rodent poison apparently originated about 1920,[1] when a company in Germany introduced a proprietary thallium rat poison. In recent years it has come into commercial use in the United States. It now appears in the form of thallous sulfate in a number of ant poisons, either in solutions with sugar or in paste form mixed with sugars, starches, greases, and absorbent material. The appearance and increasing number of these poisons have necessitated the development of an accurate method for the determination of thallium in products of this nature.

Hillebrand and Lundell[2] have published methods for the determination of thallium in inorganic compounds by weighing it as the chromate, thallic oxide, or thallous iodide. Among other proposed gravimetric

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[1] U. S. Dept. Agr. Bur. Biol. Survey Bull. **238**, p 1.
[2] Applied Inorganic Analysis, pp. 376–78.

methods are those in which thallium is weighed as thallous sulfostannate, Tl_4SnS_4; as thallous acid sulfate, $TlHSO_4$; or as the neutral sulfate, Tl_2SO_4; and as the chloroplatinate, Tl_2PtCl_6. Sikazo Nisihuko[3] has determined thallium gravimetrically as the cobaltinitrite, $Tl_3(Co(NO_2)_6)$.

Among the volumetric methods are those based on the oxidation of thallous to thallic sulfate in hydrochloric acid solution by potassium permanganate,[4] potassium bromate,[5] potassium iodate,[6] and ceric sulfate.[7] For small amounts of thallium, a colorimetric method has also been used.[8]

Of the above listed methods, the thallous iodide procedure appeared to the writer to be most promising provided conditions could be established to minimize the solubility of the precipitated thallous iodide, which is soluble to some extent in water. According to F. Kohlrausch,[9] 1 liter of water dissolves 0.0847 gram of thallous iodide at 26° C., but it is less soluble in solutions containing potassium iodide, alcohol, or a little acetic acid; and is nearly insoluble in solutions containing ammonium hydroxide or sodium thiosulfate.

A number of trials and modifications involving the oxidation of the organic matter, reduction of the thallium from the thallic to thallous condition, and precipitation of the thallium with varying amounts of potassium iodide, finally led to the development of the thallous iodide method, which is described as follows:

PROCEDURE

Weigh a quantity of the sample containing 0.1–0.15 gram of thallous sulfate (usually about 10 grams), transfer to an 800 cc. Kjeldahl flask, and add 25 cc. of concentrated H_2SO_4 followed by 5–10 cc. of concentrated HNO_3. After the first violent reaction has ceased, heat on a Kjeldahl digestion apparatus until white fumes of H_2SO_4 are evolved. Add a few drops of HNO_3 and continue the heating and addition of HNO_3 until the organic matter has been destroyed, as evidenced by a colorless or light yellow solution. Cool, add 10–15 cc. of water, again cool, and wash the contents of the flask into a 400 cc. beaker, continuing the washing until the volume is 60–70 cc. Boil several minutes to remove all the HNO_3, cool, and filter into a 400 cc. beaker. Wash with hot water until the volume in the beaker is 175 cc., neutralize with NH_4OH, and then slightly acidify with H_2SO_4 (1+4). Add 1 gram of $NaHSO_3$ to insure reduction of the thallium from the thallic to the thallous form. Heat to boiling, add 50 cc. of 10% KI solution, stir, and let stand overnight. Filter through a tight Gooch crucible containing two disks of S & S 589 white ribbon filter paper covered by a medium pad of asbestos. Wash 4 or 5 times with 10 cc. portions of 1% KI solution, and finally with absolute alcohol. Dry to constant weight at 105° C. (1-1½ hours in oven), and weigh as thallous iodide, TlI. From this weight calculate the percentage of thallium as thallous sulfate, Tl_2SO_4, using the factor 0.7616.

[3] *J. Soc. Chem. Ind.*, Japan 37, Suppl. binding 180 (1934).
[4] Hawley, *J. Am. Chem. Soc.*, 29, 300 (1907).
[5] Zintl and Reinäcker, *Z. Anorg. Elem.*, 153, 276 (1926).
[6] Berry, *Analyst*, 51, 137 (1926).
[7] *Ibid.*, 54, 461 (1929).
[8] Shaw, *Ind. Eng. Chem. Anal. Ed.*, 5, 93 (1933).
[9] *Z. Phys. Chem.*, 64, 168 (1908).

DISCUSSION

Analyses were made on some thallous sulfate-sugar solutions without destroying the organic matter. Accurate results were obtained with some mixtures, but as would be expected the precipitated thallium in most cases appeared colloidal and had a tendency to pass through the Gooch crucible during filtering and washing.

The specified amount of potassium iodide added in the precipitation of the thallous iodide is necessary, as it appears that the slight acidity of the solution before precipitation, together with the excess potassium iodide and presence of ammonium salts, renders the precipitate practically insoluble.

The presence of substances, such as silver compounds, that are soluble in sulfuric acid and precipitated by potassium iodide would interfere with the results obtained by this method. However, no samples have been encountered that contained interfering substances.

ANALYSES OF PREPARED SOLUTIONS AND SAMPLES

A 1 per cent solution of C. P. thallous sulfate was prepared at 25° C. The thallous sulfate crystals had previously been dried in an oven at 105° C., and sulfate determinations indicated a high degree of purity. At the same temperature, aliquots containing 0.075, 0.1, 0.125, and 0.15 gram, respectively, of thallous sulfate were measured from a buret; and with the exception of oxidation of the organic matter, the thallous sulfate was determined by the method described. The amounts of thallous sulfate in the different aliquots correspond to the varying amounts of thallous sulfate present in 10–15 grams of commercial ant baits. Results illustrating the recovery of the thallous sulfate are shown in Table 1.

TABLE 1.—*Recovery of thallous sulfate from water solutions*

ALIQUOT OF 1% Tl₂SO₄	EQUIVALENT Tl₂SO₄ TAKEN	TlI OBTAINED	Tl₂SO₄ RECOVERED	ERROR
cc.	*gram*	*gram*	*gram*	*per cent*
7.5	0.075	0.0984	0.0749	−0.13
10.0	0.100	0.1316	0.1002	+0.20
12.5	0.125	0.1639	0.1248	−0.16
15.0	0.150	0.1970	0.1500	0.00

With similar aliquots of the thallous sulfate solution, liquid and paste mixtures analogous in composition to certain proprietary products were prepared, and their thallous sulfate content was determined. The liquid mixtures consisted of thallous sulfate solution, two grams of honey, and three grams of granulated sugar. Two grams of honey, three grams of granulated sugar, one gram of ground walnut meat, and the varying aliquots of thallous sulfate solution were used in preparing the pastes. The results of the analysis are shown in Tables 2 and 3.

TABLE 2.—*Recovery of thallous sulfate from prepared solutions containing honey, granulated sugar, and thallous sulfate*

ALIQUOT OF 1% Tl₂SO₄	EQUIVALENT Tl₂SO₄ TAKEN	TlI OBTAINED	EQUIVALENT Tl₂SO₄ RECOVERED	ERROR
cc.	*gram*	*gram*	*gram*	*per cent*
7.5	0.075	0.0982	0.0748	−0.27
10.0	0.100	0.1312	0.0999	−0.10
12.5	0.125	0.1632	0.1243	−0.56
15.0	0.150	0.1966	0.1497	−0.20

TABLE 3.—*Recovery of thallous sulfate from prepared pastes containing honey, granulated sugar, walnut meat, peat moss, and thallous sulfate*

ALIQUOT OF 1% Tl₂SO₄	EQUIVALENT Tl₂SO₄ TAKEN	TlI OBTAINED	EQUIVALENT Tl₂SO₄ RECOVERED	ERROR
cc.	*gram*	*gram*	*gram*	*per cent*
7.5	0.075	0.0984	0.0749	−0.13
10.0	0.100	0.1310	0.0998	−0.20
12.5	0.125	0.1646	0.1254	+0.32
15.0	0.150	0.1961	0.1493	−0.47

J. J. T. Graham of this laboratory, using this method on two samples, each containing 0.1 gram of thallous sulfate and similar in nature to those mentioned in Table 3, obtained a recovery of 0.1002 and 0.0995 gram, or 99.85 per cent.

DETERMINATION OF VOLATILE FATTY ACIDS AS AN APPROACH TO THE EVALUATION OF SPOILAGE IN CANNED SARDINES

By FRED HILLIG (Food Division,* Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

In previous communications[1,2,3] report was made on the determination of volatile fatty acids in canned salmon, tuna fish and herring roe, as an approach to the problem of evaluating spoilage.

The work has now been extended to cover the determination of volatile acids in sardines (California) and the purpose of this paper is to present the facts developed.

It was found that canned sardines prepared from the freshest possible raw material contain small quantities of volatile fatty acids. As decomposition becomes more and more extensive there is a progressive increase in the amount of these acids present.

Volatile and formic acid numbers were then determined on an authentic pack of sardines. Results are given in Table 1. A description of the packs follows.

Code 2.—Packed 3-3-36, 6:00 p.m. Fish out of water 12 hours. They were in good condition, with no detectable decomposition.

Code 5.— Packed 3-4-36, 7:00 p.m. Fish out of water 37 hours. In general the eyes were fairly bright. In many fish the gills were bright on one side, but faded and darkened on the other side. The flesh was somewhat soft and had lost its translucent appearance, becoming slightly opaque.

Code 8. · Packed 3-5-36, 8:00 a.m. Fish out of water 50 hours. The eyes of the fish were reddened, the gills foul, and the flesh soft. The entire lot had a distinct odor of decomposition.

Code 14.—Packed 3-7-36. Fish badly decomposed, soft, spongy, and sour. Flesh reddened and gassy.

All packs were made at Los Angeles from the same lot of fish.

TABLE 1.- *Analysis of canned sardines*

	CODE 2		CODE 5		CODE 8		CODE 14	
CAN NO	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER	FORMIC ACID NUMBER	VOLATILE ACID NUMBER
	$mg/100\ g$	$cc\ 0.01\ N$ per 100 g	$mg/100\ g.$	$cc\ 0.01\ N$ per 100 g	$mg/100\ g.$	$cc\ 0.01\ N$ per 100 g.	$mg./100\ g$	$cc\ 0.01\ N$ per 100 g
1	1.3	15 8	1 6	21.6	3 9	57 1	15.5	156 1
2	1.2	15 8	1 7	22 1	3 3	58.1	17.5	168.2
3	1 3	15.9	1 6	22 2	5.8	60.2	18.1	170 8
4	1.4	16 4	1 5	22 5	3 9	61.7	18 3	174 2
5	1 2	16 5	1 5	22 8	4 0	65 3	19 5	181 1
6	1.4	16 7	1.8	22 8	3 9	65 3	18.4	181 8
7	1 5	16.9	1 7	23 5	5 0	66 4	18.4	183.5
8	1 3	17.1	1.7	23.6	4.6	67.8	18.9	183.5
9	1.5	17.4	1.5	23.9	5.9	71.1	18.6	189.5
10	1.8	18 6	1.7	24.5	6.0	80.8	21.1	201.5
Av.	1.4	16.7	1.6	23 0	4.6	65.4	18.4	179.0
Max.	1.8	18.6	1.8	24.5	6 0	80.8	21.1	201.5
Min.	1.2	15.8	1.5	21.6	3.3	57.1	15.5	156.1

The presence of acetic acid in sauce-packed sardines, especially mustard packs, may limit the value of a determination of volatile acidity. However, if formic acid is determined on the distillate the method becomes valuable as an index of the condition of the sample under examination. Formic acid was determined on sardines packed in mustard and tomato sauces, with the results given in Table 2.

Codes 5M and 5T were packed from the same lot of sardines as was Code 5, Table 1; likewise Codes 8M and 8T were packed from the same lot as was Code 8. It will be noticed that the formic acid obtained in Codes 5M and 8M check closely with that found in Codes 5 and 8, while

TABLE 2.—*Analysis of sardines packed in mustard and tomato sauces*

| | MUSTARD SAUCE PACK | | TOMATO SAUCE PACK | |
| | CODE 5M | CODE 8M | CODE 5T | CODE 8T |
CAN NUMBER	FORMIC ACID NUMBER	FORMIC ACID NUMBER	FORMIC ACID NUMBER	FORMIC ACID NUMBER
	mg./100 g.	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*
1	1.7	3.5	2.1	4.7
2	1.8	3.6	2.8	4.8
3	1.8	4.0	2.9	5.1
4	1.8	4.1	2.9	5.7
5	1.8	4.1	3.2	6.9
6	1.9	4.1	—	—
7	1.9	4.3	—	—
8	1.9	4.3	—	—
9	2.0	4.6	—	—
10	—	4.6	—	—
Av.	1.8	4.1	2.8	5.4
Max.	2.0	4.6	3.2	6.9
Min.	1.7	3.5	2.1	4.7

the quantities in Codes 5T and 8T show a tendency to be slightly higher. A sample of the sauce was not available for analysis, and no definite statement can be made as to its formic acid content.

The methods of analysis used were those given in a previous report.[2]

An attempt was then made to identify the individual acids comprising the acid mixture following the procedure of fractionation previously used.[2]

Since Curve 1 falls between the acetic and formic acid lines, the presence of formic acid and one or more acids higher in the series is indicated. Fractionation Curve 6 starts just below the line for propionic acid. The angle at which it crosses the propionic acid line indicates that the highest detectable member of the series of acids present is propionic acid, with possible traces of one or more of the higher members of the series.

A study of the table of rates of distillation of this series of acids up to iso-butyric acid[1] shows that 500 cc. of distillate collected under the conditions laid down will contain practically all of the N-butyric and iso-butyric acids, and that 1.5 per cent of the propionic acid and 12.5 per cent of the acetic acid will be left in the distillation flask. With these facts in mind the writer attempted to verify the above conclusion as to the presence of propionic acid. The neutralized distillates obtained in preparing the curves shown in Figure 1 were evaporated to dryness, the formic acid was destroyed, and the remaining acids were recovered as previously described.[2] A new distillation curve was then prepared (Curve 1, Figure 2). Since the curve passes between the lines for acetic and pro-
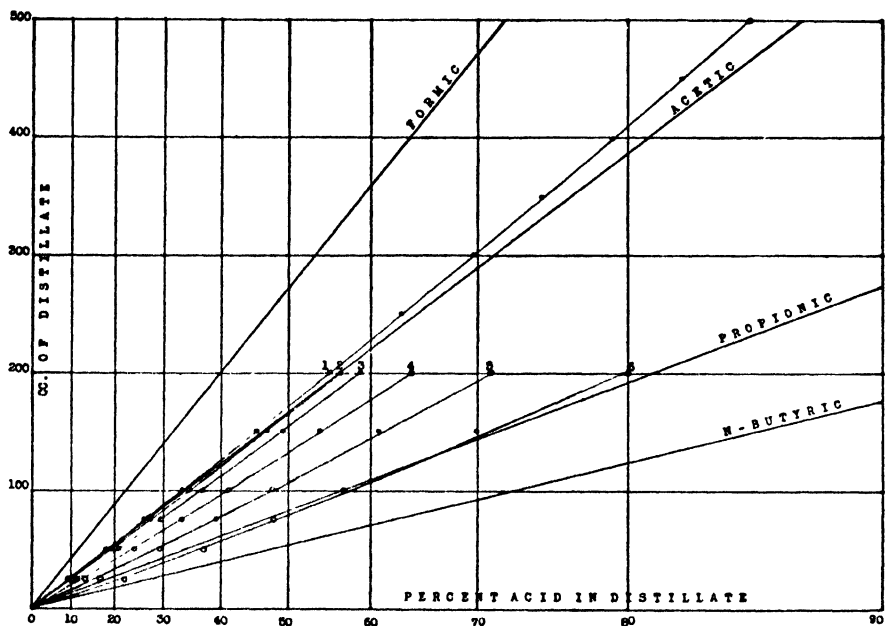
FIG. 1.
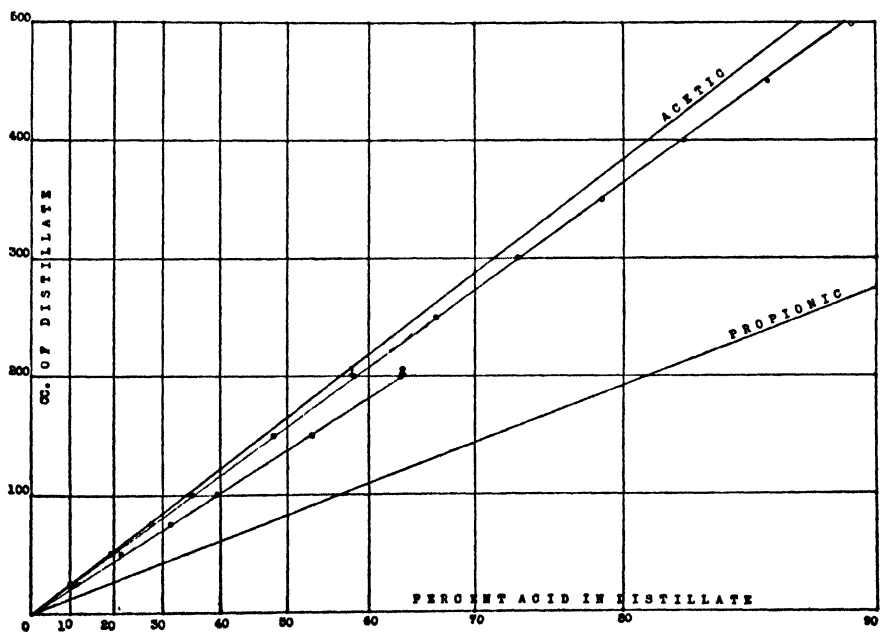
FIG. 2.

FRACTIONATION OF VOLATILE FATTY ACIDS FROM SARDINES, CODE 8

pionic acids, the presence of acetic acid and one or more acids higher in the series is indicated. Curve 2 was prepared from the distillation of the acids remaining in the flask after the 500 cc. of distillate used to prepare Curve 1 had been collected. Since the butyric acids, if present, have been practically eliminated in the first 500 cc. of distillate, and formic acid has been destroyed, Curve 2 should coincide with the acetic acid line if propionic acid is absent. The fact that Curve 2 falls materially below the acetic acid line is a further indication of the presence of propionic acid in the sample under investigation.

SUMMARY

A method for the evaluation of spoilage in canned sardines is proposed. The procedure is simple and yields diagnostic and consistent results.

THE ALCOHOLS AS A MEASURE OF SPOILAGE IN CANNED FISH

By DUNCAN A. HOLADAY (U. S. Food and Drug Administration, Washington, D. C.)

During a study of spoilage in canned sea foods it appeared that a determination of the amounts of alcohols present would be useful as an index of decomposition.

Experimental packs of fish were used for the investigation. The material ranged from fresh fish to putrid fish, and it was classified organoleptically before processing was begun. The method used for the determination of alcohols was essentially that of Friedemann and Klaas,[1] and their original paper should be studied. This method, which involves a controlled alkaline permanganate oxidation following the removal of interfering substances, is quite specific for alcohols as a group.

Table 1 shows the results that were obtained on packs of mackerel, salmon, and brine-packed sardines. Since these results may involve more than one alcohol, they are expressed as cubic centimeters of 0.02 N permanganate per 100 grams of sample. It appears that good fish contain very small amounts of alcohol, and that as decomposition proceeds the quantities present become progressively larger.

An attempt was made to identify the alcohols present. For this study, 2.6 liters of filtrate, representing 360 grams of putrid fish, were purified exactly as directed in the regular method. The purified distillate was concentrated to a final volume of 8 cc. by successive fractional distillations, and 50 per cent of the volume was collected each time. From 2 cc. of this concentrate fractionated in a micro-fractionating column about 0.05 cc. of distillate with a strong odor of ethyl alcohol was obtained. This distillate was treated with 3-5 dinitrobenzoyl chloride. After three re-

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[1] *I. Biol. Chem.*, 115, 47 (1936).

TABLE 1.—*Alcohols in canned fish*

(cc. 0.02 N KMnO$_4$ per 100 grams)

CAN NUMBER	CODE 1	CODE 2	CODE 3	CODE 1	CODE 2	CODE 3	CODE 1	CODE 2	CODE 3
	MACKEREL			SALMON			SARDINES		
1	4.2	10.0	52.3	5.0	16.4	35.4	10.2	18.9	106.8
2	3.2	17.2	57.4	3.7	7.5	62 2	4.1	18.5	75.7
3	3.4	13.3	27.6	7.1	9.8	40.5	6.5	27.3	68.9
4	3.9	8.6	50.3	5.2	14.9	60.4	3.3	12 1	91.0
5	3.6	10.9	39.5		16.4	61.7	3.8	13.6	70.6
6		8.8	39.8		6.5	46.2	6 8	16.7	65 8
7		10.6	32.3		17.2	33.4	9.0	11.6	77.0
8		8.8	70.0		10 1	25.2	6.0	12.5	73.7
9		16.8	28.5		16.7	58.7	3 8	16 1	92.3
10		18.0	30 9		10.1	52.5	5.6	22.7	117.6
Average	3.6	12.3	42.9	5 3	12.6	47.7	5.9	17.0	83.9
Maximum	4.2	18.0	70.0	7.1	17.2	62.2	10.2	27.3	117 6
Minimum	3.2	8.6	27 6	3.7	6.5	25.2	3.3	11 6	65.8

Mackerel: Packed at Terminal Island, Calif.
* Code 1 packed 10-3-34, 7 p m Fish selected for freshness Out of water 11 hours.
* Code 2 packed 10-4-34, 9 a.m Fish from the same lot as Code 1, but out of water 14 hours longer.
Fish were somewhat wrinkled, with a dull luster, some had begun to turn red around the eyes and were a trifle soft. The odor of the fish after washing was slightly sour.
* Code 3 packed 10-4-34, 6 p m. Fish from the same lot, but out of water 9 hours longer than Code 2
Fish were all bloody around the eyes, and were somewhat soft after cleaning Fish had a persistent sweetish odor of decomposition which could not be removed by washing.
Salmon Code 1 packed 7-12-35, Cordova, Alaska Fish out of water 24 hours Eyes bright, gills odorless, flesh firm.
* Code 2 packed 6-27-36, Bristol Bay, Alaska Fish out of water 45-57 hours Gills of some fish were quite white, others pink to red Eyes on the upper side slightly dull, on the under side more or less red Odor of some was sour, while others were practically odorless.
* Code 3 packed 6-28-36 from the same lot as Code 2 Out of water 62-74 hours Eyes somewhat sunken, very dull. Slime quite sour in odor. Flesh had lost its resilience but was firm. The flesh had a slightly putrid odor.
Sardines: Packed at Terminal Island, Calif.
Code 1 packed 3-3-36, 6 p.m Fish out of water 12 hours Fish were in good condition; with no detectable decomposition.
Code 2 packed 3-4-36, 7 p.m. Fish out of water 37 hours In general the eyes were fairly bright In many fish the gills were bright on one side, but faded and darkened on the other side. The flesh was somewhat soft and had lost its translucent appearance, becoming slightly opaque
Code 3 packed 3-5-36, 8 a m Fish out of water 50 hours The eyes of the fish were reddened, the gills foul, and the flesh soft. The entire lot had a distinct odor of decomposition
All packs from the same lot of fish.
* These codes are identical to the same numbered codes used by Hillig and Clark, *This Journal,* 21, 688 (1938).

crystallizations the dinitrobenzoates obtained melted at from 86° to 89° C. The melting points of some pure dinitrobenzoates are: Ethyl, 92.7°; propyl, 73°; and butyl, 62.5° C. From these data it appears that the major portion of the alcohol present is ethyl.

The method follows:

METHOD FOR DETERMINATION OF ALCOHOLS IN CANNED FISH

REAGENTS

1. *Calcium hydroxide suspension.*—Slake 100 grams of CaO (reagent quality) and make up the suspension to 1 liter.
2. *Sodium hydroxide.*—5 N. Keep in a glass-stoppered bottle *not* lined with paraffin.
3. *Potassium permanganate.*—Make up 0.1 N stock solution and dilute as needed.
4. *Sodium thiosulfate.*—Make up 0.1 N solution and dilute as needed.

Use all-glass apparatus. Two 500 cc. round-bottomed boiling flasks, a 75° angle adapter, and a Friederichs condenser, standard taper joints No. 29/42, are recommended.

PROCEDURE

Pass the entire contents of a can of fish through a meat chopper three times and thoroughly mix the material after each grinding. Weigh 50 grams of this material into a 250 cc. beaker, stir to a uniform suspension with 100 cc. of water, and transfer quantitatively to a 250 cc. volumetric flask. Add 15 cc. of 2 N H_2SO_4, mix well, and add 15 cc. of 20% phosphotungstic acid solution. Dilute the mixture to 250 cc., *shake vigorously*, allow to stand 5 minutes, and filter through a folded filter paper. Place 150 cc. of the filtrate in a 500 cc. boiling flask and add 10 cc. of Deniges' reagent (U.S.P. XI) and a few glass beads. Make to 200 cc. and distil off 100 cc. into another 500 cc. boiling flask. Add 5 cc. of Deniges' reagent to the distillate and add lime suspension until the mixture becomes *orange in color*. Shake vigorously, make to 150 cc., and distil off 100 cc., collecting the distillate in a 100 cc. volumetric flask. Pipet an aliquot into a 200 cc. Erlenmeyer flask, add 10 cc. of 5 N NaOH and 25 cc. of 0.02 N permanganate with constant rotation. Cover with a small beaker, place in a boiling water bath, and heat for at least 20 minutes. Cool, add 10 cc. of 10 N H_2SO_4, enough KI to react with the remaining permanganate, and titrate the liberated I with 0.02 N thiosulfate. Calculate the quantity (cc.) of permanganate oxidized per 100 grams of sample, neglecting the volume of solids.

Choose the aliquots so that not more than 6 cc. of permangante is consumed in the reduction. For most samples, a 25 cc. aliquot will be correct, but if a smaller aliquot is used, add distilled water to make the volume in the reduction flask up to 60–70 cc. (This volume is important. The oxidation of alcohol is not quantitative, but if the alkalinity, volume, and time are controlled, it is consistent.)

Take special care to have clean glassware. Wash all the apparatus frequently with cleaning solution and rinse with distilled water *poured from an all-glass wash bottle*. Do not allow the lower parts of the pipet to touch the hands or the desk, as any dust or organic matter will give variable results. It is advisable to plug the stems of the pipets with cotton.

Use the following factors for converting from permanganate to ethyl alcohol:

$$cc. \times 0.0855 = mg. \text{ ethyl alcohol (for 0.02 } N);$$
$$cc. \times 0.0427 = mg. \text{ ethyl alcohol (for 0.01 } N);$$
$$cc. \times 0.0215 = mg. \text{ ethyl alcohol (for 0.005 } N).$$

SUMMARY

A determination of the amount of alcohol present is proposed as a measure of the extent of decomposition of canned fish.

CHEMICAL COMPOSITION OF COTTONSEED HULL BRAN*

By D. M. MUSSER†

This work was undertaken as one phase of a broad investigation‡ of the chemical and physical properties of all constituents of the cotton

plant, with the objective of improving the economic status of the cotton industry through the development of new uses.

Cottonseed hull bran was subjected to a detailed analysis, including a spectrographic examination of the ash. An integrated chemical study of this material has never been reported in the literature, although various constituents have been determined by several investigators. Hudson and Harding[1] determined the yield of xylose obtainable from cottonseed hull bran. Markley[2] studied the non-nitrogenous components and reported analyses for furfural, xylose, cellulose, and lignin. Anderson and his associates[3,4] described the isolation of a hemicellulose and its hydrolytic products. McBryde,[5] McHargue,[6] and Sheets and Thompson[7] investigated the mineral matter of cottonseed hull ash.

EXPERIMENTAL

Analyses for the following constituents were made on a representative sample of hull bran according to the methods cited: cellulose by the chlorination procedure of Cross and Bevan;[8] lignin by the method of Ritter, Seborg, and Mitchell;[9] furfural and pentosans by the A.O.A.C. procedures;[10] hydrolysis number by the method of Hawley and Fleck;[11] and methoxyl, acetic acid, ash, moisture, ether extract, one per cent sodium hydroxide extract, cold water extract, and hot water extract, as suggested by Schorger.[12]

The cottonseed hull bran was first freed as completely as possible from hull fibers, then ground to pass a 40-mesh sieve, and finally oven-dried (105° C.). The results, which in all cases represent the average of two or more determinations, are recorded in Table 1.

Markley reported 51.7 per cent cellulose, 23.83 per cent lignin, and 25.33 per cent furfural.

In preparing samples of cottonseed hull ash for spectrographic examination, the hull material was obtained from hand-ginned seeds, and special precautions were taken to avoid contaminants. When the ashing was carried out in porcelain crucibles at a temperature of about 600° C., some variations in relative amounts of the elements were observed. Satisfactory results were obtained, however, when the hulls were ashed in platinum crucibles at a temperature of approximately 850° C.

The spectrographic analyses were made with a Hilger E-1 quartz spectrograph in the region 2450–6700 Å. The ashed samples were vaporized in a graphite arc operated from a 220-volt d.c. source at 6–12 amperes

[1] *J. Am. Chem. Soc.*, **39**, 1038 (1917).
[2] *J. Am. Soc. Agron.*, **20**, 1102 (1928).
[3] Anderson and Kinsman, *J. Biol. Chem.*, **94**, 39–47 (1931).
[4] Anderson, Hechtman, and Seeley, *Ibid.*, **126**, 175 (1938).
[5] Tenn. Agr. Expt. Sta. Bull. 4, No. 5 (1891).
[6] *J. Am. Soc. Agron.*, **18**, 1076 (1926).
[7] U. S. Dept. Agr. Farmers Bull. 1179 (1920).
[8] Doree, The Methods of Cellulose Chemistry, D. Van Nostrand Co., New York, 1933, p. 331.
[9] *Ind. Eng. Chem. Anal. Ed.*, **4**, 202 (1932).
[10] *Methods of Analysis, A.O.A.C.*, 1935, p. 344.
[11] *Ind. Eng. Chem.*, **19**, 850 (1927).
[12] *Ibid.*, **9**, 556 (1917).

TABLE 1.—*Composition of oven-dried cottonseed hull bran*[a]

	per cent
Cross and Bevan cellulose	53.40
Pentosans in Cross and Bevan cellulose	19.05
Hydrolysis number (loss in cellulose due to 15% H_2SO_4 hydrolysis)	33.40
Furfural	22.50
Total pentosans	38.40
Lignin	23.40
Nitrogen (Kjeldahl) in lignin	0.52
Total nitrogen (Kjeldahl)	0.54
Methoxyl	2.16
Acetic acid by hydrolysis	4.98
Ash	2.28
Ether-soluble	0.27
1% Alkali-soluble	20.22
Cold-water soluble	1.87
Hot-water soluble	7.52

[a] Original hull bran contained 8 11% moisture.

with electrodes of the "special, highest purity" spectrographic grade of graphite.

The spectrograms§ of the ashed hulls showed the presence of large quantities of calcium, magnesium, sodium, and potassium. In addition, iron, manganese, copper, boron, phosphorus, silicon, barium, aluminum, and traces of zinc and nickel were present.

SUMMARY

Cottonseed hull bran has been analyzed for the following constituents: cellulose, lignin, methoxyl, acetic acid, ash, furfural, pentosans, and extractives by different solvents. Spectrographic analysis of the ash is also reported.

STUDIES ON THE QUANTITATIVE ESTIMATION OF LIGNIN

IV. EFFECT OF CERTAIN PROTEINS ON THE DETERMINATION OF LIGNIN BY THE FUMING HYDROCHLORIC ACID METHOD

By MAX PHILLIPS (Industrial-Farm Products Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture)

In a previous communication from this laboratory,[1] results were presented on the action of 42–43 per cent and 5 per cent hydrochloric acid on various carbohydrates in relation to the determination of lignin by the method of Goss and Phillips.[2] In this paper results of a similar study

[1] Phillips and Goss, *This Journal*, 21, 140 (1938).
[2] *This Journal* 19, 341 (1936).

are given to show the effect on a number of proteins of the reagents used in this determination.

Several investigators have pointed out that whereas lignin isolated from wood by hydrolysis with either 42–43 per cent hydrochloric acid or 72 per cent sulfuric acid contains practically negligible quantities of nitrogen, this is not the case with lignin isolated from plants containing greater quantitites of nitrogenous constituents.

Paloheimo[3] has called attention to the fact that proteins are not completely hydrolyzed by the strong mineral acids used in the determination of lignin and he has, accordingly, suggested that from the weight of lignin obtained, be deducted the weight of the crude protein in the lignin (N×6.25). Although similar procedure has been followed in this laboratory, it has always been realized[4] that this involves the assumption that the nitrogenous complexes associated with the lignin are of protein character—a fact by no means established. It has also been fully recognized that the lignin figures thus obtained are approximations only.

Norman and Jenkins[5] found that no precipitate is obtained when egg-albumin or caseinogen is allowed to stand for 16 hours with 72 per cent sulfuric acid, and then diluted to 3 per cent and boiled. However, it was noted that when these proteins were added to straw and the mixture was treated as above described, there was an increase in the apparent lignin content of the straw. This, the authors believe, is probably due to an interaction of certain degradation products of protein with the lignin. In a later publication Norman[6] concludes that the increase in the weight of the crude lignin is caused by the condensation of the lignin with large protein fragments, partially deaminated, and that amino acids do not appreciably increase the apparent lignin content of straw.

EXPERIMENTAL

For this investigation there were used six proteins of vegetable origin: namely, zein, α-globulin of sesame seed, α-globulin of tomato seed, coagulated adsuki bean proteins, coagulated Georgia velvet bean proteins and coconut globulin, and one protein of animal origin, namely, lactalbumin. A series of experiments was first conducted for the purpose of ascertaining the effect on the several proteins studied of successive treatments with cold fuming hydrochloric acid and boiling dilute hydrochloric acid. All conditions were those prescribed by Goss and Phillips[2] for the quantitative estimation of lignin by the fuming hydrochloric acid method. The results obtained are recorded in Table 1.

It will be observed that the quantity of residual material obtained when the several proteins under examination were subjected to the

[3] *Biochem. Z.*, **165**, 463 (1925); Transactions of the Agricultural Society of Finland, Part 13, Helsiniki (1926); *Biochem. Z.*, **214**, 161 (1929).
[4] Phillips, *This Journal*, **15**, 126 (1932); **18**, 390 (1935).
[5] *Biochem. J.*, **28**, 2160 (1934).
[6] *Ibid.*, **31**, 1567 (1937).

TABLE 1.—*Results of treatment of proteins with cold fuming and boiling dilute hydrochloric acid*

PROTEIN	WEIGHT OF PROTEIN	N IN PROTEIN*	INSOLUBLE RESIDUE		N IN INSOLUBLE RESIDUE		
			WEIGHT	CALCULATED ON BASIS WEIGHT OF ORIGINAL PROTEIN	PER CENT	WEIGHT	CALCULATED ON BASIS OF ORIGINAL N PRESENT
	grams	*per cent*	*grams*	*per cent*		*grams*	*per cent*
Lactalbumin	0.5000	13.78	0.0038	0.76	5.13	0.00019	0.28
	1.0000		0.0083	0.83	4.16	0.00034	0.25
Zein	0.5000	15.15	0.0287	5.74	13.52	0.00388	5.12
α-Globulin of Sesame Seed	1.0000	16.89	0.0086	0.86	13.51	0.00116	0.69
α-Globulin of Tomato Seed	1.0000	16.36	0.0042	0.42	14.70	0.00062	0.38
Coagulated Adsuki Bean Proteins	1.0000	13.84	0.0344	3.44	12.29	0.00423	3.06
Coagulated Georgia Velvet Bean Proteins	1.0000	13.89	0.0138	1.38	12.23	0.00169	1.22
Coconut Globulin	0.5000	15.84	0	—	—	—	—
	1.0000		0.0026	0.26	17.06	0.00044	0.28

* Not corrected for moisture.

successive action of cold fuming hydrochloric acid and boiling dilute hydrochloric acid varied considerably. In the case of zein it amounted to 5.74 per cent, whereas the percentage of unhydrolyzed material was practically negligible in the case of the coconut globulin. The percentage of nitrogen in the residual material was generally less (and in certain cases considerably so) than in the original material.

The data presented in Table 2 show the effect of varying quantities of added protein materials on the yield of lignin obtained from rye straw. In all experiments there was used one gram of rye straw that had been successively extracted with a 1:2 alcohol-benzene solution, hot water, and boiling one per cent hydrochloric acid, as directed by Phillips.[7] The amounts of protein added to the one gram of rye straw were 0.1, 0.2, and 0.3 gram, respectively. In this series of experiments, as well, the conditions were exactly those prescribed by Goss and Phillips[2] for the quantitative estimation of lignin in plant materials.

It will be observed from Table 2 that, with only two exceptions, the yield of ash-free crude lignin increased with the increase in the quantity of protein added. The nitrogen in the crude lignin, calculated as per cent

TABLE 2.—*Effect on yield of lignin from rye straw of adding varying amounts of protein*
1,000 grams of rye straw used for each experiment.[1] Per cent nitrogen in the extracted straw, 0.63.

PROTEIN ADDED	WEIGHT OF ADDED PROTEIN	WEIGHT OF N IN ADDED PROTEIN	TOTAL N ORIG. ADDED	WEIGHT OF CRUDE LIGNIN (ASH-FREE)	N IN CRUDE LIGNIN				INCREASE IN WEIGHT OF CRUDE LIGNIN DUE TO ADD. OF PROTEIN	FACTOR FOR CORRECTING FOR INCREASE IN WEIGHT OF N
					CALCULATED ON ASH-FREE BASIS	CALCULATED ON ASH-FREE BASIS	INCREASE IN WEIGHT OF N IN CRUDE LIGNIN	CALCULATED ON BASIS OF TOTAL N ORIG. PRESENT		
	gram	gram	gram	gram	per cent	gram	gram	per cent	gram	
None	0	0	0.0063	0.1970	1.81	0.00356	—	56.50	—	—
Lactalbumin	0.1000	0.01378	0.02008	0.2003	2.42	0.00484	0.00128	24.10	0.0033	2.58
"	0.2000	0.02756	0.03386	0.2033	2.68	0.00545	0.00189	16.09	0.0063	3.33
"	0.3000	0.04134	0.04764	0.2071	2.79	0.00578	0.00222	12.13	0.0101	4.55
Zein	0.1000	0.01515	0.02145	0.1995	2.41	0.00481	0.00125	22.42	0.0025	2.00
"	0.2000	0.03030	0.03660	0.2067	2.78	0.00574	0.00218	15.68	0.0097	4.45
"	0.3000	0.04545	0.05175	0.2164	3.03	0.00655	0.00299	12.65	0.0194	6.49
α-Globulin of Sesame Seed	0.1000	0.01689	0.02319	0.2102	2.42	0.00509	0.00153	21.95	0.0132	8.63
"	0.2000	0.03378	0.04008	0.2170	3.06	0.00664	0.00308	16.57	0.0200	6.49
"	0.3000	0.05067	0.05697	0.2094	2.97	0.00622	0.00266	10.92	0.0124	4.66
α-Globulin of Tomato Seed	0.1000	0.01636	0.02266	0.2020	2.49	0.00503	0.00147	22.20	0.0050	3.40
"	0.2000	0.03272	0.03902	0.2105	2.87	0.00604	0.00248	15.48	0.0135	5.44
"	0.3000	0.04908	0.05538	0.2157	3.19	0.00688	0.00332	12.42	0.0187	5.63
Coagulated Adsuki Bean Proteins	0.1000	0.01384	0.02014	0.2048	2.26	0.00463	0.00107	22.99	0.0078	7.29
"	0.2000	0.02768	0.03398	0.2054	2.56	0.00526	0.00170	15.48	0.0084	4.94
"	0.3000	0.04152	0.04782	0.2167	2.67	0.00578	0.00222	12.09	0.0197	8.87
Coagulated Georgia Velvet Bean Proteins	0.1000	0.01389	0.02019	0.2032	2.00	0.00406	0.00050	20.11	0.0062	12.40
"	0.2000	0.02778	0.03408	0.2052	2.23	0.00457	0.00101	13.41	0.0082	8.12
"	0.3000	0.04167	0.04797	0.2062	2.42	0.00499	0.00143	10.40	0.0092	6.43
Coconut Globulin	0.1000	0.01584	0.02214	0.2029	2.18	0.00442	0.00086	19.96	0.0059	6.86
"	0.2000	0.03168	0.03798	0.2068	2.35	0.00486	0.00130	12.80	0.0098	7.54
"	0.3000	0.04752	0.05382	0.2001	2.45	0.00490	0.00134	9.10	0.0031	2.31

[1] Rye straw extracted successively with 1:2 alcohol-benzene solution, hot water, and boiling 1% HCl solution.

of the total nitrogen originally present in the straw and in the added protein, decreased in every case with the increase in the quantity of nitrogen in the sample. This is due, no doubt, to the fact that the proteins studied are much more susceptible to the hydrolytic action of the strong mineral acid used than are the nitrogenous complexes present in the hydrolyzed straw. It will be observed that 56.50 per cent of the nitrogen originally present in the hydrolyzed straw was found in the lignin residue, and that when proteins had been added, the results ranged from 9.10 per cent, in the case of coconut globulin, to 24.10 per cent in the case of lactalbumin. Judged by their relative resistance to hydrolysis, the nitrogenous complexes of rye straw must be considered as being quite unlike those of protein in character.

In the last column of Table 2 are recorded the ratios between the increase in the weight of crude lignin due to the addition of proteins and the increment of nitrogen in the crude lignin. It will be observed that this ratio varies both with the quantity and type of protein added. In a certain limited number of cases the ratios approach the figure 6.25, the conventional factor used for calculating the percentage of nitrogen found into percentage of crude protein, but in most instances this is not true. Because of this wide variability, it is not possible to compute the ratio between the increase in the weight of the crude lignin and the increment of nitrogen in the lignin that would be applicable in all cases. Moreover, of the total nitrogen in such materials as straws, stalks, hulls and cobs, only a portion can be considered as arising from proteins. The nature of the remaining nitrogen is, in the main, unknown. To assume, therefore, that the nitrogen in the crude lignin obtainable from such materials when analyzed by the method of Goss and Phillips is protein in character is, of course, not justified in a strict sense. When the nitrogen content of the lignin is small the error thus introduced is not appreciable. However, in determining the lignin content of materials rich in protein, it is not possible to apply a suitable correction for the nitrogenous complexes in the lignin because of the variability in the ratio between the increase in the weight of the crude lignin and the increment of nitrogen in this material. In such instances all that can be done is to report merely the weight of the ash-free crude lignin and the percentage of nitrogen in the lignin.

SUMMARY

The effect of proteins on the determination of lignin by the method of Goss and Phillips has been investigated. The results indicate that the resistance to hydrolysis of the several proteins studied (either alone or in the presence of different proportions of lignified plant material) by cold 42–43 per cent and boiling 5 per cent hydrochloric acid is quite different. Because of this variability, it is not possible to compute a fixed ratio

between the increase in the weight of the crude lignin and the increment of nitrogen in this material.

The writer wishes to express his thanks to Dr. D. B. Jones of the Protein Research Division of this bureau for supplying the proteins used in this investigation.

THE PHOTOMETRIC DETERMINATION OF NICOTINE ON APPLES, WITHOUT DISTILLATION

By L. N. MARKWOOD (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.)

In the method for determining nicotine on apples sprayed with nicotine bentonite previously described by the writer[1] the nicotine, after removal from the fruit, is distilled and converted to the silicotungstate according to conventional procedure.[2] The silicotungstic acid precipitation has been generally regarded as the most sensitive as well as the most accurate means for determining nicotine. Several micro-procedures are based on it.[3] Recently, however, a color test was described by Barta and Marschek[4] for the determination of nicotine, and was applied by them to the examination of tobacco. It shows promise of displacing the silicotungstate method for micro amounts of nicotine. When this test was described earlier by Barta[5] as a means of determining pyridine it was pointed out that nicotine also responds to the test, with the development of a red color the same as pyridine, but with a sensitivity not equal to that with pyridine. The reagents used were an aqueous solution of cyanogen bromide and an alcoholic solution of β-naphthylamine.

In view of the claim that nicotine can be determined in tobacco directly, without distillation, simply by extracting the tobacco with these two reagents, filtering, and measuring the color intensity or the absorption coefficient,[4] it seemed appropriate to investigate the possible application of this reaction to a direct determination of nicotine deposits on sprayed apples.

Barta[5] states that the solution to be tested should ideally contain only pyridine, as acids weaken the color and ammonia causes orange-yellow instead of red to appear. Obviously it is necessary to use some reagent to remove a water-insoluble nicotine insecticide from the surface of an apple, and if distillation is to be dispensed with this reagent must be considered in the color formation.

Experiments were conducted on apples sprayed with a water-insoluble nicotine insecticide (nicotine bentonite). The results show that the nico-

[1] *This Journal*, 21, 151 (1938).
[2] *Methods of Analysis*, A.O.A.C., 1935, 60
[3] T. Kosu, *J. Agr. Chem. Soc. Japan*, 7, 977 (1931); L. Nagy, *Biochem. Z.*, 249, 404 (1932); J. R Spies, *Ind. Eng. Chem., Anal. Ed.*, 9, 46 (1937); L. D. Goodhue, *Ind. Eng Chem., Anal. Ed.*, 10, 52 (1938) *Mezőgazdasági Kutatások*, 10, 29 (1937).
[5] *Biochem. Z.*, 277, 412 (1935).

tine deposit can be determined directly and accurately through the color reaction mentioned and that the method is suitable for control operations where routine analyses are to be made.

Nicotine bentonite was chosen as the insecticide for experimental work, because it is the most important of the fixed nicotines and is now coming into commercial use. The nicotine in this product is only partially soluble in water and in acid solutions, but it is brought completely into solution by a weak concentration of fixed alkali. When apples coated with nicotine bentonite are shaken in a closed container with this alkaline solution, the nicotine is completely removed. This constitutes the first step in the determination of nicotine on apples. The procedure necessary to bring the alkaline extract to the condition under which the color test can be applied is presented in this paper.

PREPARATION OF STANDARD NICOTINE SOLUTIONS

In the preparation of known nicotine solutions, which are needed for the evaluation of unknowns, it is convenient to employ nicotine bentonite as the reference material. With ordinary care this product is stable over long periods. It can readily be made by the method described by C. R. Smith.[6] The nicotine content is determined by the official method[2] and ordinarily it is 5 to 8 per cent.

Although nicotine bentonite can be dispersed in sodium hydroxide solution to bring the nicotine into true solution as the free base, the bentonite forms a colloidal suspension that is not readily filterable. Acidification or neutralization renders the suspended matter flocculent and filterable, but causes re-formation of insoluble nicotine bentonite. For example, if the alkaline suspension is neutralized with acetic acid to the phenolphthalein end point, the filtrate contains only about 70 per cent of the total nicotine. The suspension must therefore be kept alkaline to prevent any loss of nicotine on filtration. Flocculation and filterability, without reprecipitation of nicotine, can be accomplished by treatment with a suitable salt, such as calcium acetate, which in this case precipitates "calcium bentonite." The latter soon flocculates and settles out, leaving a practically clear supernatant liquid, which is readily filterable to a crystal-clear filtrate containing all the nicotine.

The flocculation of the calcium bentonite serves the further important purpose of removing the coloring matter that is brought out of apple skin by alkali, which removal is desirable in a solution that is later to be tested for light absorption.

To prepare solutions for the standard curve, a single quantity of nicotine bentonite powder is treated as outlined and the alkaline mixture is made to a fixed volume. Various aliquots of the alkaline filtrate are taken and adjusted to the same sodium hydroxide content as that of the

* U. S. Patent 2,096,566, Oct. 19, 1937. Also J. Am. Chem. Soc., 56, 1561 (1934).

largest aliquot, also the same as that of the unknown. The purpose of this adjustment with alkali is to provide a uniform concentration of sodium salt in each standard solution. In other work to be published the writer has found that the concentration of sodium acetate formed on neutralization with acetic acid influences to some extent the degree of color development.

There is still variation in the small quantity of calcium found in the standards, due to the calcium acetate, all the calcium of which is not precipitated by the bentonite. However, the intensity of color does not appear to be affected by such variations at these low concentrations, and therefore no adjustment for calcium need be made.

Color development is markedly influenced by the hydrogen-ion concentration of the solution. Strong alkalinity prevents it altogether. As alkalinity decreases, intensity of color in a given time increases until a maximum is reached at the phenolphthalein end point. Further additions of acid cause a progressive reduction in intensity. Therefore, to achieve maximum sensitivity the solution is neutralized (decolorized) to phenolphthalein with acetic acid and made to a fixed volume. A small, fixed portion of each of these standard solutions is then treated with the color-forming reagents, as described later. The coincidence of maximum sensitivity with the phenolphthalein end point is fortunate, since it is a simple matter to make this adjustment and the solution is left in a colorless condition. In practice, first a strong and then a weaker solution of acetic acid is used. To prevent the reappearance of the pink color, one drop of acetic acid beyond the disappearance of the color is added.

The color-forming reagents are an aqueous solution of cyanogen bromide and an alcoholic solution of β-naphthylamine. A definite proportion of volume of reagents to volume of test solution must be used to get the proper color (red after yellow) and the maximum intensity in a given time, that is the same conditions must prevail throughout. The reagents, 1 cc. of cyanogen bromide and 5 cc. of β-naphthylamine, should be added to 5 cc. of solution in the order stated. The color reaches a maximum intensity in 50 to 70 minutes, or about 1 hour, at which time readings are made in a suitable photometer. In the work described here there was used a neutral-wedge (visual) photometer equipped with three absorption tubes (1, 3, and 10 cm. in length) and with an approximately monochromatic blue filter (optical center at 0.49 micron).[7]

The photometer readings, when plotted against concentrations, give a straight line, in conformity with Beer's law. The mechanism of the color reaction is not fully understood.[8] Addition of the cyanogen bromide alone produces no color if the nicotine concentration is quite low, but if it is sufficiently high a yellow color soon appears. On addition of the

[7] *This Journal*, 19, 130 (1936).
[8] Kulikow and Krestowosdwigenskaja, *Z. Anal. Chem.*, 79, 452 (1930).

naphthylamine the color changes slowly through yellow and orange to red or pink.

The method follows:

(a) *Sodium hydroxide solution.*—0.5%.

(b) *Calcium acetate solution.*—Containing 4 grams of Ca per liter. This may be made by warming 10 grams of $CaCO_3$ with a dilute solution of 12–13 grams of glacial acetic acid, and diluting to 1 liter.

(c) *Phenolphthalein.*—Usual indicator strength.

(d) *Acetic acid solutions.*—A strong solution (about 30%) and a weaker solution (about 2%).

(e) *Cyanogen bromide.*—Prepared fresh before using. A fresh 10% KCN solution was added dropwise to saturated bromine water until the latter was just decolorized. The solution was then diluted to five times its volume.

(f) *β-naphthylamine.*—0.2 gram of the pure product was dissolved in 100 cc. of 95% alcohol. (This solution should be prepared fresh before use and kept away from sunlight to avoid coloration. A moderate fluorescence in direct daylight is normal.)

(g) *Bentonite.*—The powdered commercial mineral.

Four-tenths gram of nicotine bentonite was treated with 400 cc. of the NaOH solution in a 1 liter volumetric flask and allowed to stand a few minutes with occasional shaking. To this was added 400 cc. of distilled water, and then 20 cc. of the Ca acetate solution, with swirling. The solution was made to volume and mixed well. After 15 minutes, by which time the flocculent precipitate had settled, the mixture was filtered rapidly through a fluted paper. To avoid disturbing the settled precipitate on decanting, the mixture was usually first transferred to a conical flask.

Aliquots of the clear filtrate were pipetted into 250 cc. volumetric flasks. These aliquots normally were 50, 100, 150, and 200 cc., and they contained 20, 40, 60, and 80 cc., respectively, of the NaOH solution. To bring them to the same alkali content there was added 60, 40, 20, and 0 cc., respectively, of the NaOH solution. After the addition of 2 or 3 drops of phenolphthalein indicator, each solution was treated with strong acetic acid almost to decolorization and then just decolorized, plus one drop over, with the weaker acid. The solution was then made to volume. A blank was also prepared but with ordinary bentonite replacing the nicotine bentonite. In this case a single aliquot of 200 cc. was taken.

A 5 cc. portion of each standard solution was pipetted into a test tube, exactly 1 cc. of the CNBr solution was added, and the solution was mixed by swirling. Then exactly 5 cc. of the naphthylamine solution was added, and the solutions were mixed well by further swirling. The tubes were stoppered and set aside in a dark cabinet for 1 hour, when photometric readings were made.

A graph was constructed showing the relationship between concentration and photometric reading. A straight line was obtained for the range investigated, viz., 0–16 micrograms of nicotine per cc. This line passes through the blank as well as through the other points. A typical graph is shown in Figure 1.

TREATMENT OF APPLES

The sample of fruit was placed in a suitable closed container and shaken with the sodium hydroxide solution. The container may be a tin can, a glass bottle, or a bell jar having a glass plate clamped over the open end,

and should be provided with a draw-off valve. When the sample is large a tin can is the most suitable container. Since the number and size of the fruit vary, a representative procedure is outlined.

The sample of 10 mature apples was placed in the container, 200 cc. of sodium hydroxide solution was added, and the container was shaken about 3 minutes. The liquid was drawn off into a 500 cc. volumetric
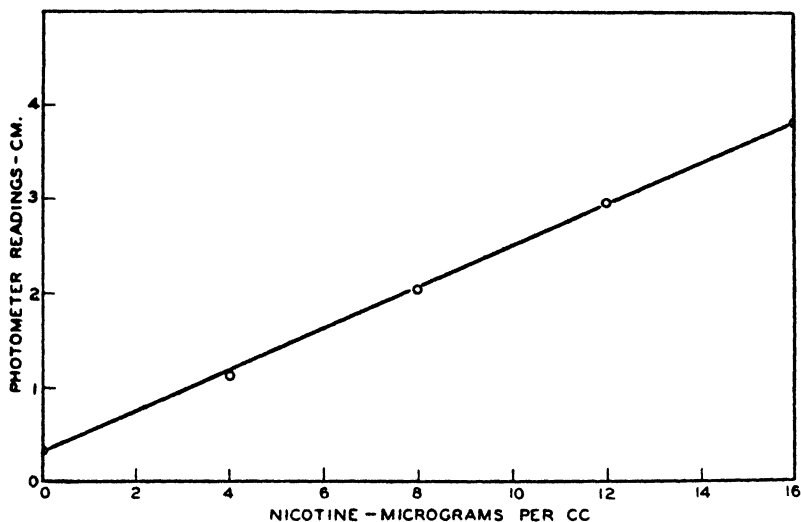
FIG. 1.—TYPICAL STANDARD CURVE FOR PHOTOMETRIC DETERMINATION OF NICOTINE.

flask. (When the apple sample is obviously too large for this amount of liquid, it will be necessary to increase the volume of sodium hydroxide solution as well as the quantities of wash water, and to adjust the final volume to the ratio shown later, viz., 500 cc. to each 200 cc. of alkali.) The apples were next shaken a minute or two, first with 180 cc. and then with 80 cc. of water, and each washing was drawn into the flask.* About 0.2 gram of powdered bentonite was then dispersed in the solution. (The object in adding this reagent here was to ensure the presence of enough bentonite for the calcium bentonite precipitate which follows.) Ten cubic centimeters of calcium acetate solution was added, then water to the mark, and the solution was mixed well. The mixture was filtered, and a 200 cc. aliquot was treated as described under "Procedure," beginning "After the addition of 2 or 3 drops." (In some cases the bentonite fails to decolorize the solution completely, leaving a slight yellowish tint, but this is of no consequence as the residual color normally disappears on neutralization. If on standing any turbidity develops in the neutralized

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* The writer is indebted to R. D. Chisholm for suggesting the following shortened manipulation in the treatment of the apples: Shake the apples with exactly 200 cc of the NaOH solution as described originally Draw an 80 cc. aliquot into a 250 cc. volumetric flask and add about 100 cc. of water, then the bentonite, etc. By this procedure washing of the apples is eliminated. It is assumed that the apple surface is dry.

solution, it may be removed by a second filtration before the color reagents are applied.)

The color test was made as described above. The concentration of nicotine in the unknown was read directly from the standard curve. Calculations were made back to the total sample with due regard for the volumes used.

When the neutral-wedge photometer[7] is used, it appears to be unnecessary to run a new set of standards for each unknown, except as an occasional check, but if a given calibration line is to be used safely with each unknown, the conditions must be uniform, especially the freshness of the color reagents and the interval allowed for color development.

Since a blank run on apples carrying no nicotine gave a reading agreeing closely with the blank of the standards an apple blank can ordinarily be dispensed with.

The sodium hydroxide solution does not give complete wetting of the waxy apple surface. When the apples are shaken with this solution, however, all the white spots of nicotine bentonite disappear, indicating that the insecticide has been effectively removed notwithstanding the apparently inadequate wetting. In comparative tests to ascertain the effectiveness of soap, which was found to give complete wetting, the recovery of nicotine was no higher when soap was used.

It is well known that apples build up a heavy coating of wax during storage, and as a result the removal of underlying spray residues, such as arsenicals, is made difficult. No tests have been made on nicotine-sprayed apples that have been kept in storage, and hence no claim is made that the method described here is applicable in such cases. This method was developed chiefly for use on fruit to be examined soon after picking.

Mature Winesap apples were used in the tests reported here. The skin was dark red and waxy.

The over-all recovery by this method was investigated by adding known quantities of nicotine bentonite to unsprayed apples. The results (Table 1) show that recovery is complete. The slight excess of nicotine recovered is without significance; it may be due to a slight concentrating effect during filtration, since the standards were filtered only once for the removal of the calcium bentonite, whereas the apple solutions were filtered again after neutralization to remove a slight turbidity.

Typical data are given in Table 2 on lots of apples sprayed at two different times (Series 1 and 2). The apples were especially sprayed for this work, and analyses were made about 4 days after spraying. A different photometer was employed in this and subsequent work, therefore no comparison of scale readings can be made with previous data.

The color reaction used is probably the most sensitive means known of determining nicotine. The sensitivity in actual use depends, of course, on the thickness of the layer of liquid being measured. By using the

TABLE 1.—*Over-all recovery of nicotine by proposed method*
(Ten apples per lot, total weight 2 pounds 5 ounces (1020 grams))

MATERIAL	PHOTOMETER READING	NICOTINE			RECOVERY
		PER CC.*	IN ENTIRE SAMPLE		
			FOUND	ADDED†	
	cm.	mmg.	mg.	mg.	per cent
Standards:					
Blank	2.11	0.00			
50-cc. aliquot	2.78	4.00			
100-cc. aliquot	3.53	8.00			
150-cc. aliquot	4.23	12.00			
200-cc. aliquot	4 92	16.00			
Apples:					
Lot 13	2.79	4.02	2.51	2.50	100.4
Lot 14	3.54	8.02	5.01	5.00	100.2
Lot 15	4.26	12.09	7.56	7.50	100.8
Lot 16	4.95	16.10	10.06	10.00	100.6

* Neutralized solution.
† As nicotine bentonite.

TABLE 2.—*Photometric determination of nicotine on lots of 10 apples*

SERIES	MATERIAL	PHOTOMETER READING	NICOTINE	
			PER CC.*	IN ENTIRE SAMPLE
		cm.	mmg.	mg.
1	Standards:			
	Blank	0.34	0.0	
	50-cc. aliquot	1.13	4.0	
	100-cc. aliquot	2.03	8.0	
	150-cc. aliquot	2.96	12.0	
	200-cc. aliquot	3.82	16.0	
	Apples: Wt. per lot, 2 lbs., 8 oz. (1133 grams)			
	Lot 2 (sprayed)	2.85	11.6	7.25
	Lot 9 (unsprayed)	0.36	—	—
2	Standards:			
	Blank	0.29	0.0	
	50-cc. aliquot	1.12	4.0	
	100-cc. aliquot	2.03	8.0	
	150-cc. aliquot	2.96	12.0	
	200-cc. aliquot	3.79	16.0	
	Apples: Wt. per lot, 2 lbs., 6 oz. (1077 grams)			
	Lot 11	2.85	11.7	7.31
	Lot 12	2.84	11.7	7.31

* Neutralized solution.

largest (10 cm.) tube with which the writer's photometer was equipped, a nicotine concentration of 1 part in 4 million, or 0.25 microgram per cc., could be determined. With a sensitivity of this order it was considered possible to determine the nicotine coverage on a single apple and this has been done.

Data for individual apples are given in Table 3. Each apple was treated with one-tenth the quantities of reagents specified for the group of 10 apples, or for the standards. The final volume for each apple was therefore 25 cc. as compared with 250 cc. for each of the standards, and this difference is taken into account in calculating the total nicotine. The results also show the uniformity of coverage that can be attained by careful spraying.

TABLE 3.—*Photometric determination of nicotine on single apples*
(Weight per apple, 4.5 ounces (127 grams))

MATERIAL	PHOTOMETER READING	NICOTINE	
		PER CC.*	IN ENTIRE SAMPLE
	cm.	*mmg.*	*mg.*
Standards:			
Blank	0.30	0.0	
50-cc. aliquot	1.18	4.0	
100-cc. aliquot	2.08	8.0	
150-cc. aliquot	2.93	12.0	
200-cc. aliquot	3.79	16.0	
Apples:			
A	3.20	13.2	0.82
B	3.18	13.2	0.82
C	3.11	12.8	0.80
D	3.13	12.9	0.81
E	3.13	12.9	0.81

* Neutralized solution.

INTERFERING SUBSTANCES

Since nicotine-sprayed fruit may also be coated with other insecticides, as well as fungicides, it becomes necessary to broaden the investigation to a study of the interference, if any, caused by these extraneous substances and means for their elimination. Accordingly, the following materials were investigated: Lead arsenate, calcium arsenate, cryolite, lime-sulfur, Bordeaux mixture, copper ammonium silicate, and a wettable sulfur containing bentonite.

In making these tests equal quantities of the supplementary material and the nicotine bentonite were used. Specifically, 0.4 gram of each was treated as described under "Procedure," that is, with 400 cc. of sodium hydroxide solution, 400 cc. of water, and 20 cc. of calcium acetate solu-

tion, after which the mixture was made to 1 liter and filtered. For convenience an aliquot of 80 cc. was neutralized and made to 100 cc. This neutralized solution was compared with a similar solution prepared from nicotine bentonite only. The results of these tests are summarized as follows:

Lead arsenate, calcium arsenate, Bordeaux mixture, copper ammonium silicate, and wettable sulfur do not interfere.

Cryolite permits a recovery of only about 84 per cent. Some cryolite is dissolved by the alkali, as shown by the appearance of a precipitate on neutralizing; moreover, fluoride ion can be detected in the solution. When this ion is removed by treating the alkaline solution with calcium oxide and filtering off the insoluble residue, the resulting solution on neutralization gives the correct reading.

Lime-sulfur (dry) causes the most interference of any added material, the recovery amounting to only about 76 per cent. The following ions can be detected in the alkaline solution: Sulfide and polysulfide (strong test), sulfate (weak), sulfite (appreciable), and thiosulfate (stronger than sulfite). Calcium is also present, but in about the same quantities as in the standards. Removal of sulfide and polysulfide sulfur (via copper carbonate) raises the recovery to 82 per cent; removal of all the forms of sulfur mentioned brings it to only 86 per cent. This is the highest recovery attained and it is still inadequate. It appears, therefore, that some factor connected with the complex nature of lime-sulfur, a full understanding of which is still lacking, is responsible for the interference. Since no remedial procedure was found, it will be necessary to separate the nicotine by distillation, submit the distillate to the color test, and compare with standards containing only nicotine in solution.

Among possible organic compounds, pyridine and its derivatives (of which nicotine is one) also respond to the color test described here. In some cases the color formed is not red; e.g., nicotinic acid produces a yellow color. Pyridine itself gives a more intense reaction than any of its derivatives, including nicotine; accordingly, if pyridine is present in a nicotine determination the results will be high. Since the simple homologues of pyridine, such as the picolines, give relatively weak colors, their presence will not seriously affect the results.[9]

The presence of pyridine or simple homologues in a spray residue is unlikely. Pyridine has been found in commercial nicotine, but it does not form so insoluble a bentonite as nicotine. Furthermore, any pyridine in water-soluble form deposited on fruit would probably soon disappear because of the volatile nature of that base. If nicotinic acid, an oxidation product of nicotine, is present, it will be removed as the insoluble calcium compound. In general, therefore, no interfering organic substances will be found in the final solution.

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[9] L. Barta and Z. Marschek, *Biochem. Z.*, 293, 118 (1937).

SUMMARY

A direct photometric method is presented for determining the amount of nicotine spray-deposit on apples. The method involves stripping of the fruit with dilute sodium hydroxide solution, purification of the extract by means of a calcium bentonite coagulate, formation of a colored compound from nicotine by means of cyanogen bromide and β-naphthylamine, and measurement of the color with a photometer. Since no distillation is required, the method is suitable for rapid mass operation.

ACKNOWLEDGMENT

A RAPID VOLUMETRIC MICRO METHOD
FOR DETERMINING ARSENIC

By C. C. Cassil (Bureau of Entomology and Plant Quarantine)
and H. J. Wichmann (Food and Drug Administration,
U. S. Department of Agriculture)

The inherent errors and limitations of the Gutzeit and other colorimetric methods for determining arsenic are generally recognized. In an effort to find a substitute, the writers have developed a rapid titration method that will determine 5–500 micrograms of arsenious oxide. It involves an arsine evolution, absorption of the arsine in mercuric chloride, and the stoichimetric oxidation of the arsine to arsenic oxide.

The principle of this method was described by Smith,[1] who showed that quantities of arsenious oxide ranging from 2 to 10 mg. could be evolved as arsine and determined with a fair degree of accuracy. Tabor[2] tried to adapt this procedure to smaller quantities but failed to obtain recoveries better than 95 per cent, principally because he worked with large volumes of solution. Winterfelt et al.[3] developed a similar procedure for quantities ranging from 50 to 500 micrograms of arsenious oxide, but their method is extremely slow, requiring a 45-minute evolution and from $\frac{1}{2}$ to 2 hours for the iodine oxidation. In some preliminary work Cassil[4] succeeded in materially shortening the time required for a determination. The method finally developed and presented in detail in this paper can be used for apple strip solutions, for material that has been submitted to a wet digestion, and perhaps for other methods of preparation.

EXPERIMENTAL PROCEDURE

ISOLATION REAGENTS

(a) *Hydrochloric acid.*—Use a C.P. concentrated acid that is arsenic-free.

[1] U. S. Dept. Agr. Bur. Chem. Circ. 102 (1912).
[2] *This Journal*, 13, 417 (1930); 14, 436 (1931).
[3] *Arch. Pharm.*, 273, 457 (1935).
[4] *This Journal*, 21, 200 (1938).

(b) *Zinc.*—Use a good grade of 20- or 30-mesh granulated zinc. It should be as free from arsenic as possible because the arsenic in the zinc predominantly determines the size of the blank.

(c) *Potassium iodide solution.*—Dissolve 15 grams of KI in water and dilute to 100 cc.

(d) *Stannous chloride solution.*--Dissolve 40 grams of As-free $SnCl_2 \cdot 2H_2O$ in 100 cc. of concentrated HCl.

(e) *Absorbing solution.*—Dissolve 1.6 grams of $HgCl_2$ (recrystallized if necessary to eliminate titration reagent blank) and 0.05 gram of U.S.P. gum arabic in water and dilute to 100 cc.

(f) *Lead acetate.*—Dissolve 10 grams $Pb(C_2H_3O_2)_2$ in 80 cc. of water, add sufficient acetic acid to have the solution just acid to litmus paper, and make to 100 cc. with water.

<div align="center">TITRATING REAGENTS</div>

(g) *Potassium iodide solution.*—Dissolve 20 grams of KI, previously recrystallized in the presence of excess iodine, in water and dilute to 100 cc. This reagent must be recrystallized in the presence of excess I_2 because most KI samples purchased on the open market have impurities which reduce iodine, thereby causing an undesirably large blank. There is no waste of KI by recrystallization, because the mother liquor can be evaporated to dryness and the recovered KI used for reagent **(c)**.

(h) *Buffer solution.*—Dissolve 10 grams of $Na_2HPO_4 \cdot 12H_2O$ in water and dilute to 100 cc.

(i) *Standard iodine solutions.*—Prepare an approximately 0.05 N stock solution of I_2 by dissolving 6.35 grams of pure I_2 and 12.7 grams of KI in a small quantity of water; filter, and dilute the filtrate to 1 liter. Proper dilutions of this stock solution are used to prepare approximately 0.001 N, 0 005 N, and 0 01 N I_2 solutions. An additional amount (25 grams liter) of KI is added to each of the dilute I_2 solutions.

(j) *Standard arsenic solutions.*—Prepare a stock solution by dissolving 1 gram of standard As_2O_3 in 25 cc. of a 20% NaOH solution. Saturate the solution with CO_2 and dilute to 1 liter with recently boiled water. 1 cc. of this solution contains 1 mg. of As_2O_3. Make three standard solutions containing 50, 250, and 500 micrograms of As_2O_3 per cc., respectively, by proper dilution of the stock solution.

(k) *Starch indicator.*—Mix about 2 grams of finely powdered potato starch with cold water to a thin paste. Add about 200 cc of boiling water, stirring constantly, and immediately discontinue heating. The solution is preserved indefinitely by the addition to the reagent bottle of approximately 1 cc. of metallic Hg.

<div align="center">APPARATUS</div>

Use a 125 cc. Erlenmeyer flask fitted with a 24/40 standard taper ground-glass joint for the generator, and attach this to an 18 cm. water-cooled condenser that has a 18/38 ground-glass joint on the upper end. Fit an adapter to the upper end of the condenser and connect the other end of the adapter to the delivery tube by means of a 10/30 ground-glass joint. The end of the delivery tube is made of methyl methacrylate resin to prevent sticking of the mercury arsenide on the inside. (Bakelite tubing has also been used satisfactorily.) The baffle on the resin tube is an aid in stirring the solution. Fill the adapter with two wads of dry Pyrex glass wool (As-free) that has been previously saturated with reagent **(f)**. (The glass wool acts as a scrubber to remove any H_2S that may be generated during the evolution. The first wad of glass wool is efficient in removing the H_2S, but it becomes saturated in about 15 determinations. The other wad acts as an indicator to show when the first wad is so spent as to need replacement with a fresh piece of impregnated glass

wool.) Construct the receiver so that the constricted end will hold approximately 1 cc. of solution in a 4 cm. length and also allow the delivery tube to extend to the bottom. The upper end of the receiving tube is approximately 25 mm. in outside diameter and about 12 cm. long. The opening in the lower end of the delivery tube should not be over 2 mm. in diameter. See Figure 2.

NOTE: A 1 mm. glass capillary tube has been used successfully in place of the methyl methacrylate delivery tube for quantities of arsenic not exceeding the equivalent of 30 micrograms of As_2O_3. If the glass tube is used, do not insert it into the absorbing liquid until immediately after connecting the generator to the apparatus, thus preventing the absorbing liquid from backing up into the delivery tube.

ARSENIC ISOLATION PROCEDURE

Place in the generator flask a suitable aliquot from the solution to be analyzed but not exceeding 75 cc., containing the equivalent of 5–500 micrograms of As_2O_3; add sufficient concentrated HCl to make the total amount of acid (H_2SO_4+HCl) approximately 10 cc., 5 cc. of the KI reagent, and 1 cc. of the $SnCl_2$ reagent, and dilute to approximately 80–90 cc. Place 1 cc. of the absorbing solution (e) in the receiver and connect it to the apparatus. Add 4–5 grams of the Zn reagent to the generator flask and connect the flask to the apparatus immediately. Bring the solution to boiling in about 2 minutes and continue the heating at approximately 95° C. until the evolution has continued 5 minutes. Approximately 1500 cc. of hydrogen is evolved in 5 minutes, and this is sufficient to expel all arsenic up to the equivalent of at least 500 micrograms of As_2O_3.

IODINE STANDARDIZATION

Three concentrations of I_2 (0.001 N, 0.005 N, and 0.01 N), are used for the ranges 5–50, 50–250 and 250–500 micrograms of As_2O_3, respectively. Standardize each I_2 solution in the same manner, i.e., add to the receiver 3 cc. of the I_2 solution from a micro buret (5 cc. graduated to .01 cc.), 2 cc. of the buffer solution, and approximately 0.5 cc. of the starch indicator, and titrate to a colorless end point, as observed through length of tube, using that strength of As_2O_3 solution (from micro buret) that is approximately equivalent to the I_2 solution being titrated. Use the delivery tube as a stirring rod. As a check on the reagents, place in the receiving tube 1 cc. of the absorbing reagent, sufficient of the KI reagent (g) to precipitate and redissolve the HgI_2, 2 cc. of the buffer reagent, 0.5 cc. of the starch indicator, and 3 cc. of the I_2 solution and titrate to a colorless end point with the As_2O_3 solution. The two titrations should check within 0.01 cc. if the reagents are pure. Determine the factor needed to transform the As_2O_3 solution into the I_2 solution. Calculate the titer of the I_2 solution as follows:

$$\frac{\text{mmg. } As_2O_3/cc. \times cc. \; As_2O_3 \; \text{soln.}}{cc. \; I_2 \; \text{soln.} \times 4} = \text{mmg. } As_2O_3/cc. \text{ of } I_2 \text{ soln.}$$

DETERMINATION

The description has been general up to this point for the range 5–500 micrograms of As_2O_3. The analyst must learn to estimate the amount of arsenic in the sample after isolation by the appearance of the suspended mercury arsenide so that the correct strength of I_2 may be used in the titration. For further descriptive purposes, it is assumed that the quantity of arsenic in the receiver tube after isolation is between the equivalent of 5 and 50 micrograms of As_2O_3.

After the 5 minute evolution, add to the receiver sufficient KI reagent (g) to precipitate and redissolve the HgI_2, and disconnect the receiver and delivery tube.

Add 5 cc. of 0.001 N I_2 solution from the micro buret through the delivery tube and stir with the delivery tube until the solution is well mixed; add 2 cc. of the buffer reagent and about 0.5 cc. of the starch indicator through the delivery tube to wash all I_2 solution into the receiver. Titrate the excess I_2 with As_2O_3 solution (1 cc. = 50 micrograms) to colorless end point. If the end point is over titrated, more I_2 solution may be added and this again back-titrated with As_2O_3 solution.

$$mmg. \ As_2O_3 = [(cc. \ I_2 - cc. \ As_2O_3 \times factor) - Blank] \times I_2 \ titer.$$

NOTE: The blank is caused solely by the arsenic in the zinc and acid used in the generator and generally amounts to 1–2 micrograms of As_2O_3, depending upon the grade of reagents used.

DISCUSSION

If pentavalent arsenic is present in the aliquot to be analyzed, it is reduced to the trivalent form by potassium iodide, which is added to the acid solution. A preliminary reduction of the arsenic oxide is not necessary, as with the Gutzeit procedure, since the generator solution is heated close to boiling as quickly as possible.

The liberated arsine is absorbed quantitatively by the solution of mercuric chloride, for which it has an unusually great affinity. The arsine in its reactions with mercuric chloride first forms one or more arsenides, but no attempt has been made to identify these compounds. They are oxidized by the excess mercuric chloride, slowly when cold and rapidly on heating, forming arsenious acid and calomel. The iodine reduction brought about by the mixture before or after the transformation represents the oxidation of arsine to arsenic oxide in which 1 As is equivalent to 8I. Equations representing the reactions involved in this method are:

(1) $As_2O_3 + 6H_2 \rightarrow 2AsH_3 + 3H_2O$.
(2) $2AsH_3 + 12HgCl_2 + 3H_2O \rightarrow arsenides \rightarrow 12HgCl + As_2O_3 + 12HCl$.
(3) $12HgCl + 24KI \rightarrow 6Hg° + 6K_2 \ HgI_4 + 12KCl$.
(4) $6Hg° + As_2O_3 + 8I_2 + 12KI + 2H_2O \rightarrow 6K_2HgI_4 + As_2O_5 + 4HI$.

FACTORS INFLUENCING THE METHOD

Since this method was intended to minimize the time required for a determination (5 minutes in its present form) sufficient hydrogen must be developed in that time to reduce the arsenic to arsine and sweep it through the apparatus into the absorption medium, where it must be completely absorbed. It has been found by experiment that 10 cc. of acid in a volume up to 90 cc. and 4–5 grams of zinc at 90°–100° C. will produce more than 1500 cc. of hydrogen in 5 minutes. This method does not limit the volume in the generator to 40 cc., as does the Gutzeit procedure, but 5–500 micrograms of arsenious oxide can be evolved from volumes up to 90 cc. It has also been demonstrated that 500 micrograms of arsenious oxide can be evolved out of a 300 cc. volume in 10 minutes by increasing the reagents proportionately. A water-cooled condenser is used in the apparatus to condense the water and acid vapor produced by boiling the solution.

Other workers have no doubt noticed the tendency of mercury arsenides to form on the inside of the glass delivery tube, but they have not commented on the strong adherence and resistance to oxidation of the deposited arsenides. Since the writers were seeking a rapid accurate method that would compete with the Gutzeit procedure, it was necessary to find some material that would not exhibit this phenomenon of sticking. The use of a tube formed from methyl methacrylate resin, known in commerce as Leucite,* obviated the trouble, probably because it is not wetted by aqueous solutions. Bakelite tubing has also been used satisfactorily. Gum arabic is added to the absorbing solution to keep the
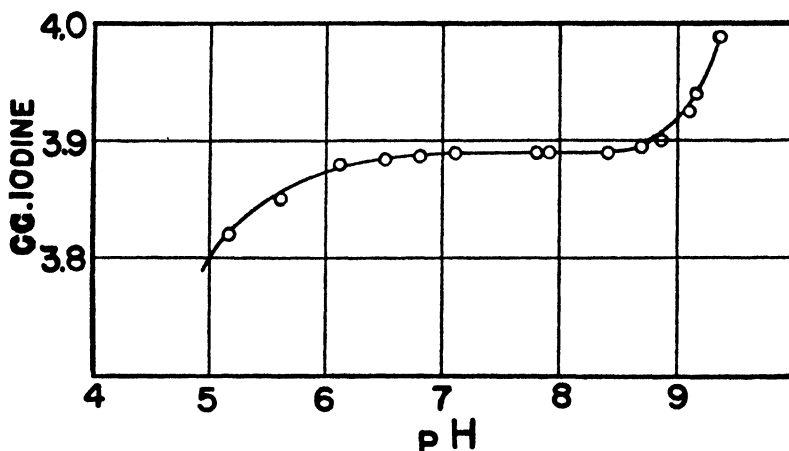
Fig. 1.—Titration curve obtained by titrating
As₂O₃ with I₂ at various pH values.

mercury arsenides in a colloidal suspension that will permit almost instantaneous oxidation by the iodine. Only 1 cc. of absorbing solution is used per determination, in order to keep the volume in the receiver small enough for the necessary micro iodine titrations. That it is sufficient to absorb the arsenic quantitatively, even though the hydrogen is passing through the receiver at the rate of 300 cc. per minute, has been proved by the complete recoveries obtained, and also by the fact that no stain was produced by the gas after passing through the solution when a stopper fitted with a Gutzeit tube and strip was inserted in the top of the receiver.

After the 5 minute evolution sufficient potassium iodide is added to form the soluble double potassium mercuric iodide, and then a standard iodine solution representing about 25 per cent excess is added to dissolve the precipitate. At this stage all the mercury is oxidized, but the arsenic is not completely oxidized until the buffer solution of disodium phosphate

* Leucite is furnished in rod form by E. I. Du Pont de Nemours and Company and the delivery tube is machined from the rod and polished. At present the complete tube is not available commercially.

is added. The optimum pH value for this buffer solution was obtained by titrating arsenious oxide solutions with 0.001 N iodine at various pH
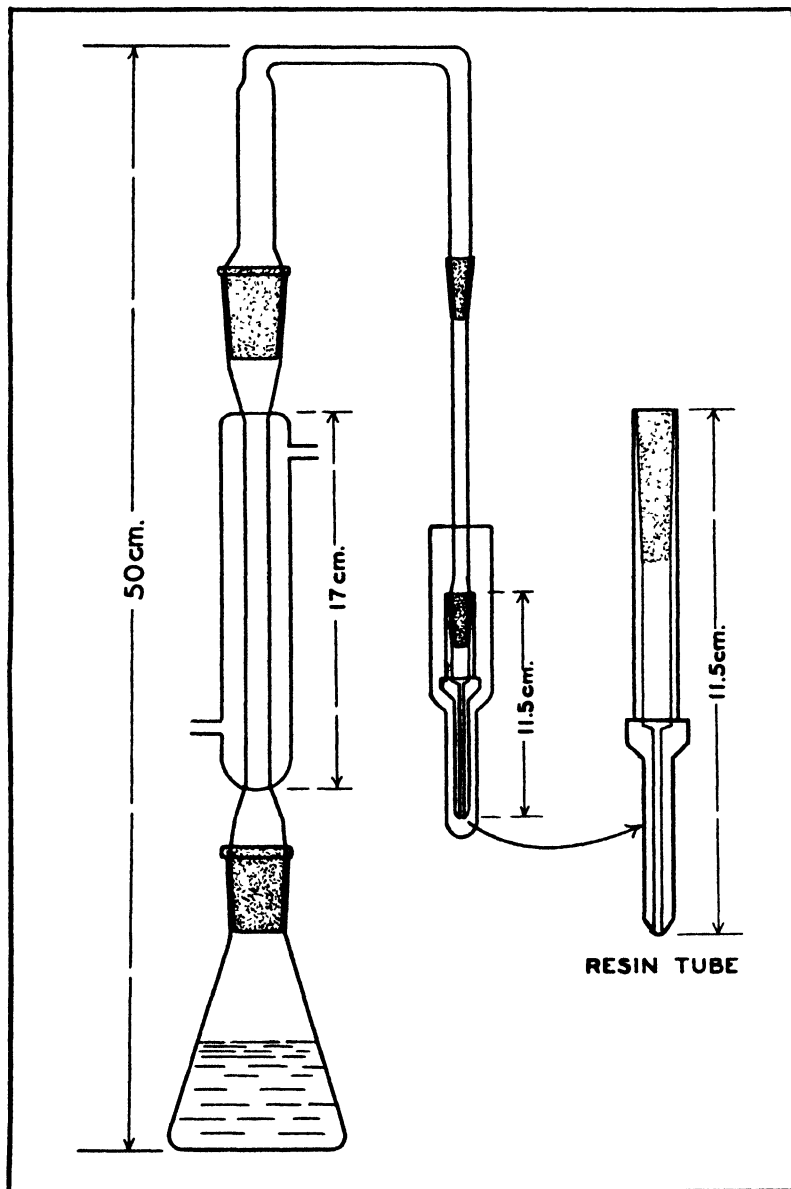
FIG. 2.—DIAGRAM OF APPARATUS USED FOR EVOLUTION,
ABSORPTION, AND TITRATION OF ARSENIC.

levels. The pH values, obtained with a Beckman pH meter after each titration, were then plotted against cc. of iodine used. As can be seen

(Figure 1) the optimum *p*H value is centered between 7 and 8.5. The reaction is decidedly sluggish at *p*H values less than 7 and the end point continues to fade. The slope of the curve below *p*H 7 depends on the patience of the analyst. Above *p*H 8.7 too much iodine is used, and it forms hypoiodites and iodides with the excess base. Disodium phosphate in the concentration used will produce a *p*H of 8.5 when no arsenic is present in the determination, but owing to the production of hydrochloric acid in the reaction the *p*H value is reduced to 7.3 when quantities of arsenic equivalent to 500 micrograms of arsenious oxide are passed into the receiving solution. As soon as the buffer has been added and the mixture stirred, the excess iodine is back-titrated with a standard solution of arsenious oxide. The quantity of arsenic can then be calculated as shown in the experimental procedure.

INTERFERENCES

Any hydrogen sulfide that may be formed during the evolution is scrubbed out of the gas as it passes through the dry plugs of Pyrex glass wool impregnated with lead acetate. Phosphides, phosphites, or hypophosphites might well produce interference if placed directly in the generator but if previously oxidized to phosphate no interference will result. Selenates and selenites are both reduced, principally to metallic selenium, in the generator. If any hydrogen selenide is formed, it is either scrubbed out of the gas by the lead acetate or it does not have any effect on the absorbing solution, because experiments have shown that quantities up to 10 mg. of sodium selenate or ammonium selenite cause no interference. Sulfur dioxide is reduced to hydrogen sulfide in the generator and either compound would interfere if it passed the scrubber. Neither substance can be present after an oxidation. Ordinary concentrated sulfuric acid may contain sufficient sulfur dioxide in 10 cc. to saturate the scrubber if not previously submitted to a wet digestion. Blanks run on digestion mixtures have proved that sulfates and phosphates do not cause any interference. If mercury salts are present in the generator, mercury is plated out on the zinc and stops the evolution of hydrogen. The only element that could possibly interfere after a wet acid digestion is believed to be antimony, and up to the present time no effort has been made to guard against this interference. Smith[1] showed that arsenic could be separated from antimony by the familiar coprecipitation of magnesium ammonium arsenate with magnesium ammonium phosphate. This separation has not been confirmed, since the analyst is seldom confronted with the need for separating these two elements. Further studies on the separation of arsenic and antimony are in progress, and it is also hoped that the procedure described can be adapted to the quantitative evolution of stibine and the iodometric determination of antimony.

Gross[5] showed that 20 mg. of pyridine or nicotine, when digested with nitric and sulfuric acids, could not be completely destroyed, and that the residue inhibited the evolution of arsine in the Gutzeit method. Cassil[6] was able to destroy these interferences by using perchloric acid in addition to nitric and sulfuric acids in the digestion. Pure pyridine or nicotine, present to the extent of 1 gram in the generator flask, exhibits the same darkening effect as that observed in the Gutzeit bottle, but on being heated the solution clears and the arsenic recovery is within the limits
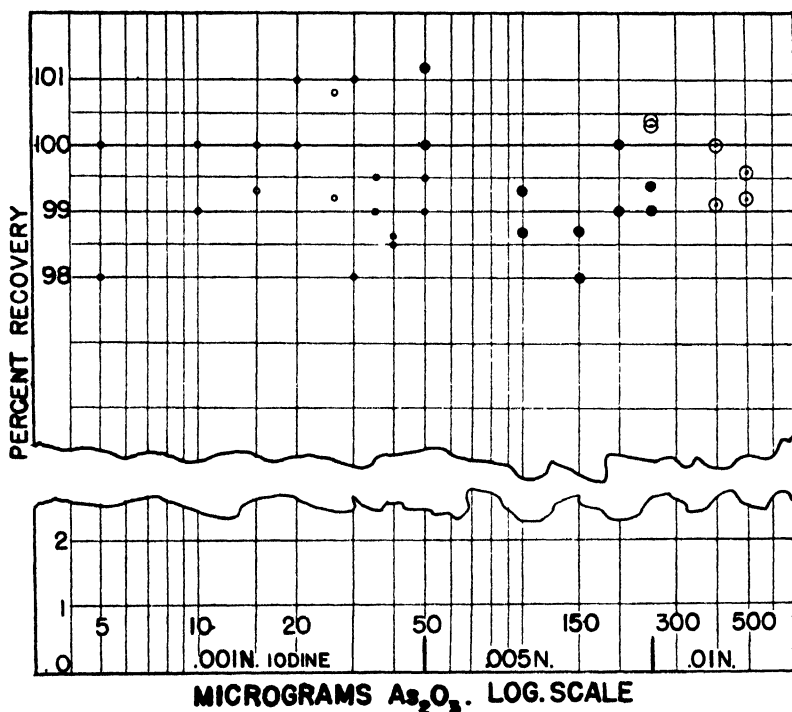
FIG. 3.—RECOVERIES OBTAINED ON QUANTITIES OF As₂O₃
RANGING FROM 5 TO 500 MICROGRAMS.

of accuracy. However, when 0.4 gram of pyridine or nicotine is partially digested with sulfuric and nitric acids and placed in the evolution flask, the arsenic recovery is only 80- 90 per cent. It is advisable to digest any organic material with nitric, sulfuric, and perchloric acids unless it can be shown that the organic matter does not interfere with the method. Rose leaves and apple plugs placed directly in the generator do not interfere with this procedure, but other kinds of organic material might produce volatile reducing constituents, not condensed or scrubbed out

[5] *Ind. Eng. Chem. Anal. Ed.*, 6, 327 (1934).
[6] *This Journal*, 20, 172 (1937).

under the conditions of the method. Analysts should, therefore, be cautious in accepting results obtained on new products without digestion unless there is proof that interfering substances are absent.

ACCURACY AND PRECISION

The results of 34 recovery experiments are given in Figure 3. The titration of the first 18 determinations on the left (small open circles) were made with 0.001 N, the next 10 (solid circles) with 0.005 N, and the last 6 (large open circles) with 0.01 N iodine. All these experiments were made with arsenious oxide that had been oxidized to arsenate by means of iodine before being placed in the generator flask. Equally satisfactory results have been obtained with known amounts of arsenic that had been submitted to an acid digestion. The average recovery shown in Figure 3 is 99.5 per cent with a standard deviation of 0.85 per cent. The minimum quantity that can be determined is set at 5 micrograms, because the titration error, 0.01 cc. of 0.001 N iodine, causes an error of more than 2 per cent on any quantity below 5 micrograms. If the analyst finds that his recoveries are consistently less than 99.5 per cent, he should check the amount of hydrogen evolved in 5 minutes and, if necessary, add more stannous chloride, heat more rapidly, or extend the evolution time a minute or two.

TIME AS A FACTOR IN CONTROL WORK

Approximately 10 minutes is required for a single determination. In routine work it is possible to run 12–15 samples per hour with two sets of apparatus, if the runs are staggered so that one is ready for titration every 4 minutes. This would amount to about 100 determinations per day. While it is true that more than 100 Gutzeit determinations can be run in a single day, considerable time is wasted in setting up standards for each set of determinations, many repeats must be made owing to the inability to choose an aliquot containing 5–30 micrograms of As_2O_3, and it is not unusual for duplicate strips not to check. Furthermore, the Gutzeit determination has a standard deviation of about 10 per cent,[7] whereas with the proposed method the standard deviation is not more than 0.85 per cent.

APPLE STRIP SOLUTION

This rapid method gives satisfactory results with apple strip solutions as prepared by the tentative A.O.A.C. method. Results obtained on the alkali-oleate strip solution, and also on a portion of the same digested with sulfuric, nitric, and perchloric acids, are shown in Table 1. For confirmatory work a group of unwashed apples and a group of washed apples purchased from a local store were used. They appear in the table as lots I and II, respectively. The oleic acid must be removed from the strip solution by the usual acidification and filtration, because its presence in

[7] Neller, J. R., *This Journal*, 12, 332 (1929).

the generator either retards or stops the evolution of hydrogen. The waxes and other organic material present in the strip solution do not interfere with the method when compared with a digested portion of the same, as shown in Table 1.

TABLE 1.—*Analyses obtained on alkali-oleate strip solutions of apples and on acid-digested portions of the same*

	STRIP SOLUTION	DIGESTED SOLUTION
	gram As₂O₃/lb.	*gram As₂O₃/lb.*
	0.0637	0.0649
	0.0631	0.0634
LOT I	0.0634	0.0631
Unwashed Apples	0.0637	0.0643
	0.0628	0.0640
Mean	0.0633	0.0639
	0.00402	0.00404
LOT II	0.00414	0.00404
Washed Apples	0.00404	0.00404
Mean	0.00407	0.00404

SUMMARY

A rapid volumetric method for determining from 5 to 500 micrograms of arsenious oxide has been developed. The complete determination after necessary sample preparation can be carried out in less than 10 minutes. The 34 results given show an average recovery of 99.5 per cent with a standard deviation of 0.85 per cent. Results are also presented to prove that the procedure is satisfactory for apple strip solutions. The main factors that make this rapid method possible are: (1) heating of the evolution solution, (2) a resin tube that prevents the mercury arsenides formed at one stage from adhering to the inside of the delivery tube, (3) the addition of gum arabic to the absorbing solution to keep the arsenides in suspension, (4) adjustment of *p*H for complete rapid oxidation, (5) the development of an apparatus and the use of an extraordinarily efficient arsine absorbent, which permits the use of the small volumes so necessary for micro titration.

THE LIMIT OF ACCURACY OF THE A.O.A.C. CHICK ASSAY FOR VITAMIN D

By BERNARD L. OSER (Food Research Laboratories, Inc., New York City)

The collaborative study of the vitamin D chick assay method of the Association of Official Agricultural Chemists, conducted in 1937 under

the associate refereeship of W. B. Griem,[1] had for its primary object the comparison of average bone ash values of tibiae of individual chicks with those obtained by analysis of composite samples representing whole groups of chicks. The results of this study have justified the practice of basing these assays on composite bone ash determinations.

The data submitted by the collaborators provided an excellent opportunity for ascertaining the practical limit of accuracy of the A.O.A.C. procedure, inasmuch as the same oils were assayed in all the laboratories and (it may be assumed) with probably more than ordinary care. It is essential that attention be focused on the statistical variation of individual bone ash values, since composite ash figures may tend to assume an absolute significance not warranted by the facts.

Through the courtesy of Mr. Griem the writer was privileged to calculate from the individual bone ash data for the assays of the U.S.P. Reference Cod Liver Oil the standard error of the mean values reported. All but one of the thirteen collaborators submitted individual, as well as composite, bone ash analyses on negative control groups and on groups receiving 10, 15, 20, and 25 A.O.A.C. units per 100 grams of feed, in the form of the Reference Oil. Included in this statistical survey are the data obtained in the writer's laboratory (designated as Lab. No. 14), which was not among those in the original collaborative study. In addition to these data, the collaborators reported the results of an assay of another sample of oil which are not included in the present analysis.

In the accompanying table are compiled the number assigned to each collaborator and, for each level of assay, the number of chicks per group, the mean ash content of the tibiae (dry, fat-free basis), the standard deviation (σ) of the individual values, and the probable error of the means (PE_m).* It will be seen that the size of the groups varied from 8 to 23 chicks, averaging 13.7; of the entire 64 groups, about two-thirds ranged in size from 12 to 16 chicks.

The maximum differences observed in individual laboratories in mean bone ash values between the negative controls and the groups receiving the highest dose of Reference Oil (25 units per 100 grams) ranged from 5.6 to 15.3 per cent; the average of these differences was 10.2 per cent.

The weighted mean bone ash values for each dosage level, also included in Table 1, spread from 33.47 per cent for the negative controls to 44.04 per cent for the 25 unit group. It is interesting to note that these weighted means (representing combined groups of 155–186 chicks) fall almost exactly on a straight line instead of on a parabolic curve such as is usually

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[1] *This Journal*, 21, 607 (1938).
* Calculated from the following equations:

$$\sigma = \sqrt{\frac{\Sigma d^2}{n-1}} \, ;$$

$$PE_m = 0.6745 \frac{\sigma}{\sqrt{n}}.$$

TABLE 1.—*Statistical variation in the averages of the individual tibia analyses as reported in 1937 collaborative study of the A.O.A.C. chick assay for vitamin B*

(Results expressed in percentage)

LAB. NO.[*]	NEGATIVE CONTROL				10/95% REF. C.L.O.				15/95% REF. C.L.O.				20/95% REF. C.L.O.				25/95% REF. C.L.O.				LAB. AVERAGES		
	NO. OF CHICKS	MEAN BONE ASH	σ	PEm	NO. OF CHICKS	MEAN BONE ASH	σ	PEm	NO. OF CHICKS	MEAN BONE ASH	σ	PEm	NO. OF CHICKS	MEAN BONE ASH	σ	PEm	NO. OF CHICKS	MEAN BONE ASH	σ	PEm	CHICKS PER GROUP	σ	MEAN PEm
1	14	34.06	3.08	0.55	15	39.59	2.20	0.38	15	42.16	2.38	*0.42*	15	41.51	2.34	0.41	13	43.98	2.03	0.38	14.4	2.406	0.428
2	15	35.99	1.31	.23	15	40.29	3.16	.65	14	43.06	2.22	.40	15	44.31	1.30	.23	14	44.74	1.05	.19	14.6	1.808	.320
3	15	36.36	1.96	.34	15	44.43	2.91	.51	15	45.10	2.91	.61	15	46.80	1.43	.25	15	47.36	0.84	.15	15.0	2.010	.352
5	16	34.39	2.53	.44	11	36.23	2.74	.66	9	39.68	3.46	.78	13	42.49	5.41	1.01	12	43.23	2.82	.55	12.2	3.392	.668
6	13	32.13	2.02	.38	13	34.86	1.87	.35	15	40.16	3.36	.59	15	43.27	1.69	.30	15	44.59	2.24	.39	14.2	2.236	.402
7	15	33.30	2.25	.39	15	35.43	2.40	.42	15	35.61	2.13	.37	15	38.88	3.34	.58	14	38.90	3.87	.70	14.8	2.798	.492
8	12	31.58	2.40	.47	11	35.50	2.79	.57	11	37.14	3.37	.68	10	38.87	2.15	.46	10	37.59	2.01	.43	10.8	2.544	.522
9	13	36.18	3.73	.69	10	44.52	2.42	.52	10	46.57	2.16	.46	11	47.55	1.75	.36	10	47.66	1.25	.27	10.8	2.262	.460
10	11	34.70	2.72	.55	13	38.90	2.54	.47	23	40.68	2.52	*.36*	11	41.79	2.12	.43	13	42.84	1.62	.30	14.2	2.304	.422
11	16	32.74	1.74	.30	13	37.71	2.66	.50	13	40.18	4.12	.77	13	42.06	4.77	*.89*	14	48.05	2.55	.46	13.8	3.168	.584
12	8	35.08	3.31	.79	9	44.91	2.21	.50	10	45.74	1.35	*.29*	9	46.31	0.81	.18	10	46.71	1.59	.34	9.2	1.854	.420
13	17	29.02	2.21	.36	15	38.79	2.57	.45	14	38.74	2.85	*.51*	15	42.60	2.73	.48	15	42.43	1.69	.30	15.2	2.410	.420
14	13	30.68	2.79	.52	22	33.12	2.44	.36	22	34.39	3.39	.50	18	40.19	4.10	.65	—	—	—	—	18.8	3.180	.510
Totals	178	—	—	—	177	—	—	—	186	—	—	—	175	—	—	—	158	—	—	—	—	—	—
Weighted Averages	—	33.47	—	—	—	38.33	—	—	—	40.29	—	—	—	42.70	—	—	—	44.04	—	—	—	—	—
Averages	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	13.69	2.490	0.462

* Collaborator No. 4 reported composite bone ash values only. Lab. No. 14 is the writer's laboratory, not included among the original collaborators.

obtained from data of this kind. Evidently the region of diminishing slope is above the 25 unit dosage level.

It is not the purpose of the writer to attempt to account for the variations reported by these collaborators, but rather to point out that such factors as strain of chicks, nutritional heredity, housing conditions, temperature control, dissection technic, etc., are undoubtedly contributing factors.

In the last three columns of Table 1 are given for each laboratory the average number of chicks per group, the mean standard deviation of the individual bone ash values, and the corresponding mean PE_m for all dosage levels. The latter figures fall within rather narrow limits (0.320–0.668) considering the variations in the average size of the groups. No consistent trend was noted in the direction of less variation within groups at either the higher or lower dosage levels. From these mean values, the standard deviation of individual bone ash values for all laboratories at all feeding levels of vitamin D was found to be ±2.490, which would indicate that the chances of a bone ash value for an additional chick in any group falling within ±2.49 per cent of the previously determined mean for that group are about 2 to 1. The mean PE_m for all laboratories at all levels was ±0.462, the average group consisting of 13.69 chicks.

However, rather than use these figures as the basis for calculating the minimum significant difference in mean bone ash between two groups of chicks, it is perhaps preferable to recognize the existence of a region of maximum sensitivity in the curve of response relating bone ash to vitamin D dosage. Most of the collaborators found the 15 unit level to fall within this range. In Table 1 the figures for σ and PE_m at the point of greatest slope on the curves of response are italicized. From these values the mean was found to be ±3.056. For convenience it may be assumed that in ordinary assays 10 chicks per group are used. In that case the standard error (or the standard deviation of the mean) would be $3.056/\sqrt{10} = 0.966$, indicating that the chances of the mean bone ash value of a second group of 10 chicks falling within ±0.966 of the original mean are approximately 2 to 1.

Using this value for the standard error (ϵ), the analyst may compute the significance of differences between mean or composite bone ash values. The standard error of such a difference is obtained from $\sqrt{\epsilon_1^2 + \epsilon_2^2}$, in which ϵ_1 and ϵ_2 are the standard errors of the individual groups. Hence in groups of 10 chicks, where ϵ_1 and ϵ_2 are 0.966, $\epsilon_{\text{difference}} = 1.366$. When a difference is equal to its standard error, it is statistically significant with a degree of certainty of 68.26 per cent, i.e. the chances favoring significance are about 2 to 1. Obviously a conclusion as to an assay can hardly rest secure on a 1 in 3 chance of such a difference being without significance. It is customary in quantitative bioassays to adopt some degree of certainty, usually based on the nature of the tests, as the criterion for evaluation of data. As stated by Dunn:[2] "The boundary lines of signifi-

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[2] *Physiol. Rev., 9,* 275 (1929).

cance should be determined by the individual experimenter with reference to his particular problem. For instance, suppose he were interested in establishing the dosage of a lethal drug, he would not wish to come within one chance in a million of giving a lethal dose to a patient due to the sampling variation in drug effect. However, if he were gambling on a horse at the race track he might be only too glad to have a chance of 60 to 40 in his favor." For a difference to be significant with a degree of certainty of 95 per cent or greater, it should be at least twice its standard error. If 99 per cent certainty is required, the difference must be 2.576 times its standard error; in this case there would be only one chance in a hundred that the difference was not significant. This somewhat severe criterion has been adopted by the British Pharmacopoeia for the official interpretation of vitamin assay data.

However, in the present writer's opinion, the less conservative degree of certainty represented by 2ϵ is better suited to the interpretation of vitamin assays and, in fact, has wider usage in this country. On this basis, then, it may be stated that the minimum significant difference in bone ash between two groups of 10 chicks each would be 2 times 1.366 or 2.73 per cent.

The increase in precision that results from using larger assay groups stands in inverse relationship to the square root of the size of the group; for example, in order to double the precision (or cut in half the minimum significant difference) the assay groups must be quadrupled. This is illustrated in the following table, which shows, for example, that in order for a difference of one per cent in composite bone ash between an assay and reference control group to have statistical significance the groups should consist of at least 75 chicks.

NUMBER OF CHICKS PER GROUP	MINIMUM SIGNIFICANT DIFFERENCE IN MEAN (OR COMPOSITE) BONE ASH
	per cent
10	2.73
20	1.93
30	1.58
40	1.37
50	1.22
75	1.00
100	0.86

It may be added that if the mean ϵ for all groups were used in this computation instead of the mean ϵ for groups at the level of maximum sensitivity, the minimum significant difference for groups of 10 chicks would fall close to 2.0 per cent bone ash. Furthermore, while the statistical analysis here presented is in the nature of a cross-section of the variability prevailing in the laboratories that participated in the collaborative study, for accurate interpretation individual assayers should subject their own data to a similar analysis.

THE QUANTITATIVE ADAPTATION OF THE CODEINE TEST TO THE COLORIMETRIC DETERMINATION OF SELENIUM IN PLANT MATERIALS*

By Jehiel Davidson (Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.)

Selenium as a plant constituent belongs to the so-called "rare" elements. While in certain limited areas the selenium content of plants is relatively high, in some plants reaching a concentration of 0.5 per cent,[1] this element is generally present in plants, if at all, in minute quantities, which are commonly estimated as parts per million. In such cases colorimetric methods are preferable to gravimetric or volumetric methods.

Two color tests are available for the qualitative detection of selenium, viz., the reaction using codeine and that using pyrol,[2] but thus far no colorimetric method for the quantitative determination of selenium has been developed.†

The codeine test, which is well known, has long been used as a qualitative procedure for the detection of selenium in sulfuric acid[3] and in glass,[4] and vice versa, selenium has been used by pharmacologists as a qualitative test for codeine.[5] When codeine is added to a solution of selenium in concentrated sulfuric acid there is produced a characteristic color varying from green to blue.

Horn[6] adapted the codeine test to the qualitative detection of selenium in plant materials and protein fractions. The material is digested in Kjeldahl flasks with sulfuric acid and mercuric oxide, as in the procedure for nitrogen determinations, and to the clear sulfuric acid digest codeine sulfate is added. It was the object of this work to adapt Horn's procedure to the quantitative determination of selenium in plant materials.

DIGESTION OF MATERIAL

It is generally a simple matter to digest 1 or 2 grams of plant material in the Kjeldahl procedure for the determination of nitrogen, but in the determination of selenium, due to the small quantity present, it is often necessary to digest as much as 10 grams, and as a result there is often serious difficulty with foaming. This difficulty was overcome (1) by using unground material whenever possible, as in the case of grain; (2) by cutting off for a short time the external source of heat after the digestion was

[1] Miller and Byers, *J. Agr. Research*, 55, 59–68 (1937).
[2] Berg and Titelbaum, Festschrift zum 70 Geburtstag von Hofr. Prof. Dr. Friedrich Emich, Emil Haim & Co., Wien and Leipzig, 1930, pp. 23–28.
[3] *J. Pharm. Chim.*, 11, 261–62 (1900).
[4] *J. Soc. Glass Tech.*, 11, 386–93 (1927).
[5] U. S. Pharmacopoeia, 1926, p. 114.
[6] *Ind. Eng. Chem. Anal. Ed.*, 6, 34–5 (1934).

well started (in exceptional cases it was necessary to cut off the external heat several times); and (3) by using large Kjeldahl flasks (800 cc.).

VOLATILITY OF SELENIUM

The volatility of selenium is influenced by two factors, its concentration and the form in which it is present. In boiling 10 mg. of selenium with hydrochloric acid in an Erlenmeyer flask on an open flame for 3 hours Brückner[7] observed a loss of 27 per cent. By continued dropwise addition of hydrogen peroxide during the boiling with hydrochloric acid the loss of selenium was prevented. Similarly, by boiling for 6 hours 10 mg. of selenium with nitric acid, which is itself an oxidizing agent, no loss of selenium resulted. By boiling 20 mg. of selenium with sulfuric acid under similar conditions the same author observed a loss of 4 per cent in 2 hours and of 10 per cent in 3 hours.

Under the conditions of the present experiments no loss could be detected when 0.05–0.2 mg. of selenium (the concentrations commonly used for the standard solutions) was digested with sulfuric acid and mercuric oxide in Kjeldahl flasks for 2.5–3.5 hours, but there were indications of some loss, not greatly exceeding the limits of experimental error, when 0.8–1.0 mg. was digested under the same conditions. It was found that the mercuric oxide used as a catalyst to hasten digestion also reduced the volatility of selenium, probably by keeping it in an oxidized state.

To prevent or minimize errors due to the possible volatilization of selenium, however, the standard solutions should be as nearly as possible of the same concentration as that of the unknown samples and should be digested for the same length of time. It is also advisable to adjust the quantities of sulfuric acid used so that the residual quantity of acid after digestion will be about the same in all determinations.

SELENIUM-CODEINE COLOR

A serious difficulty in the development of the codeine test into a quantitative procedure was the changeability of the selenium-codeine color.

The presence of too much water, for example, prevented the initial development of the color or destroyed it when the water was added after the color development. When solid codeine sulfate was added to a sulfuric acid solution of selenium the development of the color was delayed and the shade was irregular, but when the codeine was added in solution a normal characteristic color began to develop immediately.

The shade of the selenium-codeine color is variously described in the literature as green or blue or as passing from blue to green. An explanation of this discrepancy suggested itself in the course of this investigation. When codeine was added in excess of the quantity required to react with the selenium in the solution the color was blue and it lasted for a compara-

[7] *Z. anal. Chem.*, **94**, 306–22 (1933).

tively long time without undergoing much change, but if insufficient codeine was added to react with all the selenium the color was green and less stable.

When the codeine was added to the sulfuric acid shortly after digestion, the color did not develop properly and was not stable, but turned to varying shades of olive. This difficulty, which was probably due to the incomplete precipitation of the mercuric sulfate, was overcome by allowing the digestates to stand overnight. The greater part of the mercuric sulfate precipitate came down immediately after cooling of the digestate, but when allowed to stand overnight an additional fine precipitate settled.

The mineral elements present in the plant material may also affect the shade of the selenium-codeine color. This difficulty was overcome as follows:

1. The standard solutions were digested with samples of a selenium-free material identical or similar in nature to the one that was to be analyzed, equal in weight to the corresponding unknown samples.

2. The standard solutions were digested with the selenium-free ash of weights of the same material that was to be analyzed equal to those of the corresponding unknown samples. Practically all the selenium was expelled during the combustion of substances that yielded an ash with an acid balance. Substances that yielded an ash with an excess of bases over acids retained a considerable part of their selenium content. It was found that when, previous to ashing, sufficient monopotassium phosphate was added to the material to give the ash an excess of acid-forming over base-forming elements, and when the ashing temperature was raised to 700° C., selenium was completely expelled from all materials. (The selenium-free ash should be added to the standard solutions toward the end of their digestion period, as it generally causes considerable bumping.)

3. Approximately as much iron and potassium phosphate (potassium and phosphorus generally constitute the greater part of most plant ashes) were added to the standard as were present in the samples of the material that was to be analyzed. Iron affected the shade of the selenium-codeine color to a greater extent than did any other ash constituent.

It was found in this investigation that iron not only does not interfere with the codeine test for selenium, as was shown by Horn,[6] but that it is essential to the proper development and stability of the color, as it is essential in the colorimetric qualitative test for selenium with pyrol.[2] It was also found to be necessary to add iron to plant materials of low iron content, such as wheat, when smaller samples were used. In all cases reported here the addition of phosphate and iron was sufficient to obtain normal results. The other two ways of overcoming interference of mineral elements (1 and 2 above) did not prove to be of particular advantage.

Occasionally for some unknown reason, such as a contamination with some organic substance, a solution failed to develop the normal color.

The solution was then returned to the Kjeldahl flask, redigested till clear, and treated anew with the codeine reagent.

Care must be taken to add sufficient codeine reagent, because it was found that several times as much codeine as selenium is required for the reaction.

INTERFERENCE OF VANADIUM

Of the mineral elements that may be present in plants only vanadium interferes with the determination of selenium under the conditions of this procedure, as then vanadium develops with codeine a color similar to that developed by selenium. Vanadium, however, seldom has been found in plants.[8] Moreover, vanadium was found to be about one-tenth as sensitive to codeine as selenium. For example, 100 micrograms of vanadium gave but a feeble test with codeine in 25 cc. of concentrated sulfuric acid. Accordingly, a plant material containing 10 p.p.m. of vanadium will cause a slight interference (about 1 p.p.m.) only when 10 grams of sample is used.

If, under exceptional conditions, the occurrence of vanadium in plants in relatively large quantities is suspected, its interference with the colorimetric determination of selenium may be overcome by making use of the fact that under the conditions under which vanadium remains in the ash selenium is expelled, as described above. It was done in two ways:

1. A weighed quantity of the material was ashed with monopotassium phosphate at about 700° C. to expel the selenium. The vanadium in the ash was determined in terms of selenium (with a selenium standard) and its value subtracted from the selenium value obtained in the ordinary procedure.

2. A sample of the same weight as the one to be used in the ordinary procedure was ashed with monopotassium phosphate and the ash containing the vanadium was added to the standard solution just before digestion was completed. (In this case the selenium concentration of the standard solution must be approximately the same as that of the sample of the substance that is analyzed.)

INTERFERENCE DUE TO TURBIDITY

Grains as a rule give a clear solution after the digestates are allowed to stand overnight. The clear liquid can then be decanted or pipetted off without disturbing the precipitate. On the other hand, the digestates of the vegetative parts of plants often remain turbid for a long time and the precipitate is readily disturbed during decantation or pipetting. In such cases filtering is necessary. A Gooch plate was placed in a small funnel and covered with wet asbestos. The stem of the funnel was put through a one-holed rubber stopper, which was placed in a suction flask. The digestates filtered readily with the aid of suction and the filtrates were per-

[8] Robinson, Steinkoenig, and Miller, U. S. Dept. Agr. Bull. 600, 1917.

fectly clear. (The same asbestos may be used two to three times after being washed each time by suction with sulfuric acid. Eventually the asbestos becomes clogged with mercuric sulfate and with the precipitated plant constituents and filtration becomes very slow.)

At times the solutions were apparently clear and did not seem to require filtration, but when placed in the colorimeter they were found to have a slight turbidity, which interfered with the readings. The solutions were then removed from the colorimeter, filtered, and returned to the colorimeter for comparison, as the filtration did not affect the selenium-codeine color.

Preparation of Standard Solutions

A selenium stock solution was prepared as follows: 1 gram of elemental selenium was dissolved in concentrated nitric acid, evaporated nearly to dryness on a steam bath, dissolved, and made up to one liter with water. The standard solutions containing 20–200 micrograms were prepared from the stock solution by dilution with distilled water.

Choice of Colorimeter

While Nessler tubes may be used for comparison of the unknown with the standard solutions, the use of a colorimeter was found to be more convenient and to give more accurate results.

Only a colorimeter whose receptacles are not attacked by sulfuric acid should be used. In good daylight a Schreiner colorimeter was found to be very convenient. A Klett colorimeter has the advantage in that it may be used with artificial light.

Range of Concentrations

When the sulfuric acid digestion of materials and standards was completed there remained in the Kjeldahl flasks 20–25 cc. of sulfuric acid solution, and by the time all digestates were made up to volume the latter reached 25–30 cc. Under these conditions the smallest quantity of selenium that could be determined by this method with a reasonable degree of accuracy was 10 micrograms. To overcome this limitation with materials of low selenium content large samples may be used. With 10 grams of material, for example, 10 micrograms of selenium would make 1 p.p.m. No upper limit need be set as the sulfuric acid digestates may be freely diluted with concentrated sulfuric acid before or after the development of the color. Too large quantities of selenium in the samples, however, may increase the danger of possible loss by volatilization and should be avoided.

The concentrations most convenient for color comparison in a colorimeter are from 2 to 8 parts of selenium per million parts of sulfuric acid.

Procedure

The plant material was digested with sulfuric acid and mercuric oxide (0.7 gram) in Kjeldahl flasks as in the nitrogen procedure. (When small samples of a material poor in iron are used it is advisable to add iron to the Kjeldahl flasks before digestion as stated under digestion of standards.) The weight of the samples depended upon the selenium content of the material. (If possible, the samples should contain not less than 20 micrograms of selenium, as those containing less are difficult to handle. A convenient sample should contain 50–200 micrograms.)

Ten grams of plant material (the maximum quantity that can be conveniently digested in this procedure) required about 75 cc. of sulfuric acid in order to have a residue of 20–25 cc. of sulfuric acid solution when digestion was complete; 5 grams of plant material required about 60 cc. and two grams about 25 cc. of sulfuric acid. The running over of the Kjeldahl flask was prevented by using unground material, by using large flasks, or by cutting off at intervals the source of external heat. The digestion was continued till digestates were colorless when cooled (2.5–3.5 hours).

The standard solutions (dilutions of the stock solution containing 20–200 micrograms of selenium) were digested with samples of selenium-free material similar in kind and equal in weight to those in which selenium was to be determined. (If such selenium-free material is not available, the ash of samples of the material that is to be analyzed, comparable in weight to that of the unknown samples but freed from selenium by ashing the material with monopotassium phosphate at about 700° C., is added to the standard solution shortly before the close of the digestion period.)

In most cases, however, it was sufficient to add to the standard solution before digestion 1 cc. of a 0.5 per cent solution of ferrous sulfate and 1 cc. of a molecular solution of mono- or di-potassium phosphate. The ferrous sulfate was dissolved with the aid of a few drops of sulfuric acid. In all cases the standard solutions were digested with sulfuric acid for the same length of time as were the unknown samples.

When selenium-free plant material was used with the standard solutions they were digested with the same quantity of sulfuric acid as was used for the samples of the analyzed materials; in the other cases they were digested with 25 cc. of sulfuric acid.

When digestion was complete the residual contents of the Kjeldahl flasks were cooled and made up to the same volume (about 30 cc.) with concentrated sulfuric acid, returned to the same flasks, stoppered, and allowed to stand overnight. Aliquots of about 20 cc. were then pipetted off into small Erlenmeyer flasks without disturbing the precipitate. If the supernatant liquid was not clear or could not be pipetted off without disturbing the precipitate, it was filtered in the manner described previously. Five to six drops of a 2 per cent aqueous solution of codeine sul-

fate or of the codeine alkaloid (the latter dissolved with the aid of a few drops of sulfuric acid) were added slowly, with constant shaking, to the solutions in the Erlenmeyer flasks, which were then stoppered and allowed to stand for about 2 hours. (For selenium solutions containing more than 200 micrograms more codeine must be added. The quantity of codeine added must be not less than 30 times the quantity of selenium present in the solution.)

The unknown solutions were compared with the standards in a Schreiner or Klett colorimeter.

RESULTS

The method was tried with varying quantities of selenium added to selenium-free wheat. Some of these results are given in Table 1. The

TABLE 1.—*Comparative colorimetric readings of duplicate standard solutions and recovery of selenium added to wheat*

SELENIUM ADDED TO STANDARDS AND WHEAT	COLORIMETRIC READINGS OF STANDARDS			RECOVERY OF SELENIUM FROM WHEAT			
				COLORIMETRIC READINGS		RECOVERY	RECOVERY AVERAGE
	1	2	AGREEMENT	STANDARD	WHEAT		
micrograms			per cent			per cent	per cent
				a*	20	20	100	
150	21	20	95	b*	20	20	100	100
				a	30	31	97	
100	31	30	97	b	30	32	94	95.5
				a	36	32	112	
50	39	36	93	b	36	32	112	112
				a	40	39	97	
20	40	40	100	b	40	38	95	96

* Duplicate determination.

agreement between duplicate standard solutions as well as between duplicate determinations of selenium added to wheat is good. The recovery of selenium added to wheat fluctuates around 100 per cent, above as well as below, which indicates that there was no loss of selenium from wheat as compared with the standard solutions. On the basis of 10 grams of wheat the concentrations given in this table will make 15, 10, 5, and 2 p.p.m., respectively.

In Table 2 are given results obtained with varying quantities of a selenium-containing flour. The variation in the quantities of selenium found

TABLE 2.—*Selenium in varying quantities of a selenium-containing flour*

WEIGHT OF SAMPLES	COLORIMETRIC READINGS		WEIGHT OF SELENIUM	AVERAGE	RELATIVE WEIGHTS OF SELENIUM
	STANDARD[1]	UNKNOWN			
grams			*m.mg.*	*mmg.*	
10.0	30	16	190		
10.0	30	16	190	190	100
7.5	30	20	150		
7.5	30	19	160	155	81
5.0	—	— —			
5.0	30	30	100	100	53
2.0	10	26	38		
2.0	10	27	37	37.5	20

[1] Standard = 0 1 *mg.*

by analysis is fairly closely related to the variation in the size of the samples.

In Table 3 are given results obtained on four samples of wheat grown under field conditions on a soil containing selenium. The analysis was repeated twice on two of the samples and thrice on two others. The duplicates and repetitions agree fairly well.

TABLE 3.—*Selenium in different samples of wheat*

SAMPLES	1[1]		2[1]		3[2]	
	P.P.M.	AV.	P.P.M.	AV.	P.P.M.	AV.
A	28				26	
A	24	26			24	25
B	24				22	
B	22	23			24	23
C	14		14		14	
C	16	15	18	16	12	13
D	22		22		22	
D	22	22	—	22	22	22

[1] 10 grams used.
[2] 5 grams used.

In Table 4 results by the present method are compared with those obtained by a method developed by Robinson and co-workers.[9]

The Robinson method is turbidimetric for the lower concentrations and gravimetric for the higher concentrations. The material for analysis, wheat and wild vegetation, was obtained from areas in which the soil contains selenium in varying concentrations. The selenium content of

[9] Robinson, Dudley, Williams, and Byers, *Ind. Eng. Chem. Anal. Ed.*, **6**, 274 (1934).

the material varied from 0 to 600 p.p.m. The results by the Robinson method were obtained by R. A. Osborn in single determinations. The results by the proposed method are all averages of duplicates. These results are offered simply as a basis of comparison of those by the proposed method with those on a series of samples available at the time the investigation was conducted.

TABLE 4.—*Comparison of results obtained by the proposed method with those obtained by Robinson and co-workers' method*

MATERIAL	PROPOSED METHOD	ROBINSON & CO-WORKERS' METHOD[1]
	p.p.m.	*p.p.m.*
Winter Wheat (plants) A	2.5	None
Winter Wheat (plants) B	13.5	10
Winter Wheat (plants) C	6.0	3
Wheat (heads)	10.0	8
Wild Aster A	137.0	110
Wild Aster B	440	350
Wild Aster C	201	174
Wild Aster D	620	620
Aster (Kuhnia)	None	None
Prairie Shoestring	12	7
Not Identified A	115	111
Not Identified B	123	156

[1] Results obtained by R. A. Osborn.

SUMMARY

The qualitative codeine test was adapted to the quantitative colorimetric determination of selenium in plant materials. It was found that the presence of iron is essential for the development of a stable and relatively lasting color.

Consistent results were obtained with this method in recovering selenium added to wheat and in determining selenium in varying weights of a selenium-containing flour. Consistent results were also obtained in determining selenium in different wheats grown in an area in which the soil contains selenium.

Results obtained with this method on wheat and on various wild vegetation grown on a seleniferous area compare satisfactorily with results obtained with another method developed by Robinson and coworkers.[9]

BOOK REVIEWS

Humus—Origin, Chemical Composition, and Importance in Nature. By SELMAN
A. WAKSMAN. 526+VIX pps. 44 text. figs. 63 tables. 2nd ed. 1938. Williams
and Wilkins. Baltimore. Price $6.50.

The author attempts, with considerable success, to present a comprehensive
discussion of humus. He reviews the historical development of concepts concerning
"humus and humic acids," explains in some detail the formation and chemical char-
acteristics of humus in soils, composts, peat, and coal, and discusses its relation to
plant nutrition, soil genesis, and soil conservation. The argument is supported by
many analytical data and copious quotations from other investigators.

The author has inserted a number of improvements, although in the main this
edition is not greatly different from the first edition that appeared in 1936. There is
still a tendency to repetition, e.g., the chapter "Humus and Soil Conservation" is
composed largely of material given in previous chapters.

As one might expect from such a distinguished microbiologist, the chemical
and especially the biological aspects of humus formation and decomposition have
been treated most comprehensively. In properly stressing the important role of the
micro-organisms, he may underestimate the importance of certain significant chem-
ical reactions that accompany the main decomposition processes. It is likely that his
specialized experience in the laboratory has determined his approach to the subject,
predominately analytical. Thus some of his concepts will probably not be entirely
acceptable to soil scientists dealing with soils as geographic bodies supporting native
plants or as fields and ranches producing crops and pastures. One point of probable
disagreement lies in the concept of humus itself. The author states, for example,
" . . . it (humus) should be used to designate the organic matter of the soil as a
whole", whereas most soil scientists consider humus to be a relatively stable product
of decomposition. Even the author himself states elsewhere, "Humus is a product
of decomposition of plant and animal residues. . . . " The author's not always defi-
nite use of these two entirely different concepts—(1) humus is the total organic
matter of the soil, and (2) humus is a special portion of the total resultant from
microbial decomposition—makes it difficult for the reader to determine when he
means one and when the other.

The author mildly criticizes the soil scientist for not giving soil organic matter
proper weight in soil classification and genesis. He then places the "Podzols, brown
forest, red, and yellow soils" in the same class as far as "abundance and nature of
humus" are concerned and the "chestnut soils" with the "serozems," although the
"brown grassland soils" are included with "chernozem"!! Such generalizations
emphasize the difficulty of specialty coordination.

While the author has not been entirely successful in coordinating his data with
those from other branches of soil science, his emphasis upon the need is timely and
cogent. It is hoped that in subsequent editions particular attention may be given
to the nature and functions of humus in different soils and to "humus" or organic
soil amendments and their effects on soil structure and plant growth.

Points where differences of opinion may occur, while possibly serious to the
individual reader, should not be overemphasized. The book is clearly the best on
the subject, serves to bring together a large body of literature dealing with organic
matter, and will be indispensable as a reference book for students and investigators.
The data on the constituents of various types of humus are especially valuable.
The bibliography includes 1,608 references and there is an author and subject-
matter index.—CHARLES E. KELLOGG.

Plant Growth-Substances. By Hugh Nicol, 108 pp. Distributed by Chemical Publishing Company of New York. 1938. Price $2.00.

The author describes experiments that led to the discovery of naturally occurring plant hormones and presents a brief and technical account of chemical methods used in the synthesis of some compounds known to be effective as plant growth-regulators. Methods used by various workers in applying growth-regulating compounds to plants are discussed, together with a detailed description of how some synthesized compounds can be used to stimulate root growth of cuttings. A chapter is devoted to the natural occurrence of growth substances and a none too clear account of the chemistry of some synthetic compounds in relation to plant growth. The final chapter deals briefly with the identification of some substances known to be effective as plant growth-regulators and clearly shows the need for more adequate chemical methods of detecting and estimating the amounts of these compounds in plant tissues.

The book should be of particular interest to those desiring a brief account of the chemistry of natural and synthetic growth-regulating substances and of how some of these compounds have been utilized in horticultural practices.—John W. Mitchell.

The Standardization of Volumetric Solutions. By R. B. Bradstreet, with a Foreword by Harry L. Fisher. 119 pp. Chemical Publishing Co. of New York, Inc. Price $3.00.

While there is a large number of comprehensive volumes on volumetric analyses, there are fewer books that deal with only the standardization of volumetric solutions. From the foreword it is learned that the purpose of this volume is to have available a ready reference of concise procedures.

The directions for standardization in this volume are contained in four chapters. Under each solution directions are given for its preparation and standardization. The reaction involved and the necessary calculations are also presented.

In all well accepted practices for the standardization of a volumetric solution the substance used in determining the strength of the solution is weighed. In this book too many of the procedures for standardization direct that the normality be determined by checking with another standard solution. In those instances where the primary standard is weighed, too small a weight is directed to be used.

For example, in the standardization of sodium thiosulfate (p. 53), 0.12 gram of potassium bromate is weighed. It is obvious that if only weights of Class S accuracy are used the errors in weighing might be greater than 0.1 per cent.

It is also fundamental practice that the primary standard substance by which the strength of the standard solution is determined must be pure or its degree of purity must be known. In only few cases does the author present detailed directions for the purification, or methods for testing the purity, of the primary substance used in the standardization.

Under the standardization of sodium hydroxide with acid potassium phthalate (p. 38), no mention is made that this salt may be obtained from the National Bureau of Standards especially prepared for use in standardizations.

Further, with respect to sodium hydroxide solution, the directions do not include the protection of the solution from carbon dioxide from the air. The caution that standard alkali solutions should be kept in alkali-resistant glass is also omitted.

Even though the literature citations appear to be entirely adequate, it is the reviewer's opinion that the worth of this book to the chemist is not commensurate with its cost.—R. L. Vandaveer.

The Structure and Composition of Foods. By ANDREW L. WINTON and KATE BARBER
WINTON. Volume IV. Sugar, Sirup, Honey, Tea, Coffee, Cocoa, Spices, Extracts,
Yeast, Baking Powder. 580 pp., 134 illustrations, John Wiley & Sons, Inc.,
New York. 1939. Price $9.00.

This fourth and final volume completes a series on the structure and composi-
tion of foods by these authors. Reviews of the previous volumes have appeared in
This Journal, Vol. XV, p. 500. Vol. XVIII, p. 647; and Vol. XXI, p. 157. The same
treatment is afforded the subjects as was followed in the preceding volumes. A
general discussion of the physical and chemical properties of sugars introduces Part
I on saccharine products. The products of sugar cane and beet, sorghum and maple
are treated in this section, together with honey, invert sirup, starch sugar, and
glucose, and caramel, to mention the chief subjects. What are called the alkaloidal
products—maté, tea, cocoa and chocolate, coffee, and other minor plants—are the
subjects of Part II. Spices and extracts, so inclusive as to be too numerous to list,
comprise Part III, to which one-half of the volume has been devoted. Those inter-
ested in the chemistry of spices have long awaited such a comprehensive compila-
tion. In Part IV in the presentation of leaven, yeast is dealt with from its modern
chemical aspects.

The sense of accomplishment must be most satisfying to these authors with the
completion of the whole work, by which they have urged "recognition of food his-
tology as the logical, although novel, approach of the student to food science and
of the trained chemist to food research of a more fundamental character than the
mere diagnosis of commercial doubtfuls and unknowns, in which field the authors
long labored." This work is a monument to those labors, representing, as it does,
the only American compilation of its kind, and rivaling works of renowned standing
from European sources. While much of the data and information is of American
sources, the choice does not neglect that from the world at large.

The reviewer can always add comment on some subjects which, to his mind,
have not been so completely treated as he believes they might have been, but why
do so when these authors have so obviously shown, by their addenda on vitamins,
an appreciation of the impossibility of bringing together in the pages of a single
book the ever-changing development of knowledge.

As would be expected from Dr. Winton, who for so long was engaged in the work
of enforcement of the Federal Food and Drugs Act, the problems of the regulatory
chemist are not overlooked, even to the concept of the newer Food, Drug, and Cos-
metic Act of 1938, as evidenced by the quotation in legal phraseology in the dis-
cussion of caramel: " . . . make the product appear better or of greater value than
it is."—HENRY A. LEPPER.

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WILLIAM JOHN GASCOYNE, 1856-1938

WILLIAM JOHN GASCOYNE

William John Gascoyne died of pneumonia at Johns Hopkins hospital December 27, 1938, after a short illness. He was the son of Elizabeth Wilson and William R D Gascoyne, an officer in the British Royal Artillery, and was born in Glasgow, Scotland, October 12, 1856.

His studies in chemistry were pursued at the University of Edinburgh, Scotland, where he received the degree of Doctor of Philosophy, and later he became an assistant to Stevenson MacAdam, F R S E He came to America and established himself as a representative of a laboratory apparatus supply house operating from New York In 1883, he was appointed State Chemist of Virginia, which position he held until 1887, when he organized the firm of Gascoyne & Company, public analysts, with headquarters in Baltimore His laboratory, library, and papers were destroyed in the great Baltimore fire of 1904.

He is survived by his wife, who was Miss Lucilla Clary, of Petersburg, Va ; three daughters, Mrs. H. E Bucholz, Mrs Kenneth F Love, and Mrs J H. Wagner; and by one son, William J Gascoyne, Jr.

Dr. Gascoyne was an active participant in the meeting of Agricultural Chemists held at Atlanta, Ga , May 15, 16, 1884, and in the Philadelphia Convention of September 8 and 9, 1884, where the Association of Official Agricultural Chemists was organized. At this meeting he was appointed a member of the Committee on Potash and of the Executive Committee He also attended the second meeting, and at the third he was appointed on the Nominating Committee and on the Committee on Nitrogen. At the fourth meeting, held in 1887, he was elected Vice-President, but he was not elected President for 1889 because he had retired from official work.

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However, he attended a majority of the meetings, including the last five, as he was much interested in the work of the Association, and enjoyed meeting his friends of the "Old Guard" and making new acquaintances. His life was given, primarily, to the study and analysis of agricultural materials.

Dr. Gascoyne was a member of the American Chemical Society, Society of Chemical Industry, American Institute of Fertilizer Chemists, American Oil Chemists Society, American Society for Testing Materials, and the National Fertilizer Association.

With the passing of Dr. Gascoyne, there remains only one of the charter members of the A.O.A.C., Dr. Charles W. Dabney.

<div align="right">H. B. McDonnell</div>

TUESDAY—MORNING SESSION

REPORT ON VITAMINS

By E. M. Nelson (U. S. Food and Drug Administration,
Washington, D. C.), *Referee*

As the work of the Associate Referees on Vitamins progresses it is
desirable to take inventory of their accomplishments and also to review
progress of other organizations in the development of methods so that
the efforts of this Association may be coordinated and attention directed
to the most urgent problems.

There is no doubt that the usefulness of the spectrophotometric method
for the determination of vitamin A will be much greater in the near
future. It is now being used extensively, but some fundamental studies
are necessary before it can be used to the best advantage. Until very
recently pure preparations of vitamin A have not been available and
therefore the exact biological value of the pure compound has not been
determined in International or U.S.P. units. Until this has been done it
is impossible to translate directly spectrophotometric readings into vita-
min A units.

The U.S.P. Vitamin Committee recognizes the value of the spectro-
photometric method in establishing the vitamin A content of cod liver
oil, but believes further collaborative studies are necessary before an
official method is adopted. A subcommittee of that organization is now
planning such a study. The American Drug Manufacturers Association
has also appointed a committee, with Charles L. Barthen as chairman,
which now has collaborative studies in progress.

The number of investigators in this field is rather limited and with the
investigations just mentioned under way it seemed inadvisable for this
Association to initiate studies this past year. For the present it seems that
the Associate Referee, J. B. Wilkie, can serve as a clearing house for in-
formation on developments in the field. Also, until some decision is
reached by the United States Pharmacopoeia regarding the acceptance
of a spectrophotometric determination for vitamin A, it is not clear
whether or not this Association should attempt to establish an official
method.

Studies on vitamin B_1 have also become more closely related to the
work of the U. S. Pharmacopoeia than had been anticipated. Last year
O. L. Kline presented a method for the determination of vitamin B_1,
This Journal, **21, 305,** and later he was appointed by this Association
as Associate Referee on Biological Methods for the Vitamin B Complex.
The method that Kline presented has been subjected to collaborative
study by the U. S. Pharmacopoeia Vitamin Committee during the past
year; very satisfactory results were obtained, and the method is now

being considered for adoption by the U. S. Pharmacopoeia. If it is adopted by that organization, there is no need for its duplication in A.O.A.C. methods. There will, however, be a need for some modification of this method or for some method better adapted to the assaying of foods of low vitamin B_1 potency. In his report Kline will present some data having immediate bearing on this subject (see p. 662).

Two methods that are now being studied appear to be wholly problems of this Association. Reference is made to the determination of vitamin D in milk, and to the same determination in products for poultry by the use of baby chicks. The Referee is indeed appreciative of the splendid cooperation received from a large number of collaborators on both of these methods. Considerable experience has shown that the method for determination of vitamin D in milk is fairly satisfactory. Certain alternatives, which do not appear to have an important bearing on the accuracy of the results, are permitted, but from the standpoint of enforcement procedures it would be well to eliminate them. Walter C. Russell, Associate Referee, is giving these matters his attention.

The Referee has given considerable attention to Biological Methods for Determination of Vitamin D Carriers because of the desirability of adequate control in this field and of developing this method to its greatest efficiency. There are at least five States using this method in controlling the quality of vitamin D carriers for poultry, and the Food and Drug Administration is also conducting an extensive survey of interstate shipments of these products. Since questions arose concerning the interpretation of certain sections of the text of this method of assay it has been entirely rewritten. Some minor changes have also been proposed both in the basal ration and in the procedure of assay.

It is with considerable regret that the Referee announces that W. B. Griem has tendered his resignation as Associate Referee. He has contributed a great deal of his time and effort to this project, and has been very successful in obtaining the cooperation of others in developing the method to its present status. The Association owes him appreciative thanks.

Recent developments indicate the desirability of developing a method for the determination of vitamin K. This term was proposed by Henrik Dam to refer to a substance which is necessary to prevent a type of nutritional deficiency in growing chicks. In the absence of vitamin K there is a delay in the clotting time of the blood, which condition is associated with low prothrombin content of the blood. Clinical studies have now led to the conclusion that post-operative hemorrhages, which so frequently occur in patients with obstructive jaundice, can be controlled to a marked degree by the proper use of vitamin K preparations. Patients with obstructive jaundice show a low prothrombin, just as chicks do when fed a vitamin-K-deficient ration. It is recommended that H. J. Almquist

of the University of California, who has contributed much to the knowledge of this vitamin, be appointed Associate Referee on Vitamin K to study the desirability of adopting a method.

The recommendations of the respective associate referees are approved.

REPORT ON VITAMIN A

By J. B. WILKIE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Several important problems relative to the spectrophotometric determination of vitamin A were indicated at this meeting last year. These problems include the investigation (a) of the destruction of vitamin A as a basis for its determination, (b) of separable fractions of fish liver oils relative to their respective spectrophotometric characteristics, and (c) of crystalline vitamin A to determine its correct E value with the hope of obtaining better correlation of all methods for determining vitamin A. The need for improvement and standardization of equipment specially suited for the purpose as well as for collaboratove work to establish the accuracy and reliability of spectrophotometric equipment by means of a stable inorganic solution was also emphasized.

Without in any way detracting from the importance of these problems, it now appears to the Associate Referee that immediate concerted action should be directed to the checking of instruments against a suitable standard with the hope of accounting for the spread in extinction coefficient values for the U. S. P. Reference Oil that has been reported from various laboratories. E values for this oil ranging from 1.4 to 1.79 have been more or less consistently obtained and reported.

It appears that Smith, Stern, and Young[1] may have the correct explanation for such discrepancies. They present data to show the effect on the absorption value of the different solvents used in vitamin A preparations, and state that with alcohol taken as 100, cyclohexane was 97.5, ether 107.5, and chloroform 89 in absorption value.

The differences between alcohol and cyclohexane are scarcely beyond experimental error, but certainly the chloroform value of 89 appears beyond experimental error. This was conclusively proved by the investigators above mentioned, who examined the same material first in a chloroform solution and in an ether solution after drawing off the chloroform in a vacuum. They found that chloroform lowered the absorption value, and that it was restored in large part in the ethereal solution These same authors also mentioned the fact that the absorption value of an oil could be increased or decreased by 40 per cent through irradiation, but that it would practically return to normal after a period of dark storage.

[1] *Nature*, 141, 551 (1938).

These phenomena were accounted for by the authors by assuming isomeric reversible changes presumably of a cis-trans type. It thus appears that the values 1.4 to 1.79 for the U. S. P. oil may likewise be attributed to such changes if the oil has been subjected to different storage conditions or if different solvents have been used.

In this laboratory the discrepancies in absorption values on raw oils having various storage periods and conditions could be explained by assuming isomerism. If such isomerism is the interfering factor, means should be sought to recognize it and avoid its effect upon the absorption. While nothing very pertinent to the solution of this problem can be offered at this time, it appears that by boiling with alcoholic potassium hydroxide as is usually done in obtaining the nonsaponifiable material for the determination, isomerism might be regulated to bring the vitamin A to some constant condition and thereby make more uniform results possible. However, this is contrary to available results, which have indicated more uniform absorption values from the raw oil than from the nonsaponifiable portion.[1] These more variable results may be caused by variations in the preparation of the nonsaponifiable fraction previous to the spectrophotometric examination. At the present time, in this laboratory, small volumes of water are being used for rinsing (each rinse amounting to only about 1/15 of the volume of the ether extract) in place of the more strongly alkaline rinses recommended previously. More vigorous agitation is also used. These rinses are diluted with about 75 cc. of water, and after very slight agitation they are discarded. This procedure sometimes necessitates the use of a drop or two of alcohol in the final rinsed extract to eliminate traces of turbidity. However, with this modification troublesome emulsions are avoided and higher absorption values are obtained than with the more strongly alkaline rinses previously recommended.

As a basis for checking over-all spectrophotometric performance, the use of a single inorganic solution was recommended last year. Conditions made collaborative work impossible, but some work pertinent to this matter was initiated. Several compounds were tried, but none seemed as promising as potassium chromate diluted with 0.05 N potassium hydroxide, which has been the subject of careful investigations by Von Halbon[2] and by Hogness.[3] Each of these investigators used a photoelectric system that admittedly is capable of greater precision and, with care, of greater accuracy than is the photographic method generally used for the vitamin A determination. Hogness reported 20 determinations of the molecular extinction coefficient of potassium chromate at 366 mu. Using his value for molar extinction coefficient obtained at a molar concentration of 2.96×10^{-5} and a 2 cm. cell, the Associate Referee calculated absorbency

[1] *J. Am. Pharm. Assoc.*, 26, 525–540 (1937).
[2] *Z. physik Chem.*, 100, 208 (1922).
[3] *J. Phys. Chem*, 41, 379–415 (1937).

or density values over a range possible by the photographic method and the use of a 1 cm. cell. The table shows the experimental data obtained compared with those calculated from the coefficient published by Hogness. The experimental values at all of the concentrations are seen to be in substantial agreement with the calculated values.

The average experimental molecular extinction coefficient of the Associate Referee differs from the Hogness value by only 2.4 per cent, while the average deviation from the mean for all of the concentrations is only 4.8 per cent. When it is considered that some of these concentrations are not the optimum ones for the making of photographic spectrophotometric determinations the agreement is seen to be very good. These data thus substantiate a recommendation for the use of potassium chromate over

Dilution-absorption value for K_2CrC_4 at 366 mu
(0.05 N KOH used as solvent)

CONCENTRATION		MOLECULAR EXTINCTION COFFFICIENT AT 366 MU		ABSORBENCY OR DENSITY		TRANSMITTANCY		SPECIFIC ABSORPTIVE INDEX OR EXTINCTION COEFFICIENT $E^{1o/o}_{1cm}$	
PER CENT	MOLS PER LITER	HOGNESS CELL 2 CM. CONC −2.96 ×10⁻⁵	EXPTL. VALUES	CALCU-LATED	EXPTL.	CALCU-LATED	EXPTL.	CALCU-LATED	EXPTL.
3.88 ×10⁻³	20×10⁻⁵	4720 ± 10	5000	.945	1.00	12	10	244	258
3 10 ×10⁻³	16×10⁻⁵	4720±10	5120	.755	0 82	17.5	15	244	264
2.33 ×10⁻³	12×10⁻⁵	4720±10	5000	.566	0 60	27	25	244	258
1.55 ×10⁻³	8×10⁻⁵	4720±10	4900	378	0 39	42	40	244	250
.077×10⁻³	4×10⁻⁵	4720±10	4500	.189	0 18	64	65	244	234
.038×10⁻³	2×10⁻⁵	4720±10	4500	0945	0 09	79	80	244	232
			Av. 4836					Av. 249	

Experimental molecular extinction coefficient checks Hogness experimental value within 2 4%.
Extinction coefficient maximum deviation from mean =7%.
Extinction coefficient average deviation from mean =4 6%

an entire range of concentration for checking over spectrophotometric performance. It is therefore recommended[1] that the instruments being used in collaborative work be checked against a suitable potassium chromate solution immediately preceding and after each vitamin A determination. The concentrations and absorption values of the standard test solutions should be reported with the data on the oils being studied.

Collaborative studies relating to the spectrophotometric determination of vitamin A are now being undertaken by the American Drug Manufacturers' Association as well as by the Vitamin Committee of the U. S. Pharmacopoeia. In order to avoid unnecessary duplication of effort it is

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 55 (1939).

therefore recommended that collaborative work directed by the A.O.A.C. on this phase of the problem be temporarily delayed.

Regardless of this temporary cessation of collaborative activity by the A.O.A.C., the several problems that were mentioned at the beginning of this report are very pertinent, but it now appears that such work may be carried out most effectively, at least in the preliminary stages, by individual effort.

REPORT ON VITAMIN D*

PRESENT STATUS OF THE USE OF THE TENTATIVE METHOD— FEEDING OF NON-VITAMIN D MILK WITH THE REFERENCE OIL

By WALTER C. RUSSELL (New Jersey Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

A tentative method for the assay of vitamin D milk was adopted at the 1936 meeting of the Association and was published in *This Journal*, 20, 78. At the 1937 meeting a revision of the tentative method was approved and was published in *This Journal*, 21, 90.

Since the principal features of the tentative method have been in force for over two years, and since several options are allowed, it seemed advisable to ascertain what options are being used and what experience collaborators have had with them.

Accordingly, a questionnaire was sent to 32 collaborators known to be concerned with the assay of vitamin D milk, and replies suitable for tabulation were received from 18. The portions of the replies that might be of interest to those concerned with the assay of vitamin D milk are summarized in the following paragraphs.

Depletion Period.—The age limits for starting the depletion period are given as 21 to 30 days. Ten assayers start animals on the depletion period at not less than 21 nor more than 26 days of age. The range of depletion periods was from 18 to 25 days, and 11 collaborators reported depletion periods of less than 21 days.

Assay Period.—Eleven assayers are using the 7-day and seven, the 10-day assay period. Supplements, both reference oil and milk, are fed for 1, 2, 3, 5, and 6 days, by those who employ the 7-day period and for 1, 3, and 8 days, by those who use the 10-day period. The results obtained with any of these procedures are apparently satisfactory if the oil and milk are fed in the same manner. Two of the 18 assayers mix the supplements with the feed. Those who use the 7-day period report the line test to be satisfactory. Some, however, find it necessary to increase the quantity of supplement ordinarily used for the 10-day period, in order to obtain a satisfactory response at 7 days.

Although more assayers are using the 7-day than the 10-day period there is no uniformity as to the number of feedings made for either period. It is possible that in some laboratories it will be difficult to feed enough irradiated milk in 6 days to obtain a satisfactory line at the end of a 7-day period, and it may be necessary to consider for adoption an official method which would permit a 7- to 10-day feeding period, with the stipulation that the reference substance and the sample are to be administered according to identical procedures.

TABLE 1.—*Effect of feeding skim milk or non-vitamin D whole milk with the reference oil*

| | | REFERENCE OIL | | REFERENCE OIL PLUS MILK[1] | |
		QUANTITY FED U.S.P. UNITS	AV. RESPONSE	QUANTITY	AV. RESPONSE[2]
				cc.	
Laboratory A 6-day feeding 7-day period		3.3	0 55	28.8 (reconstituted skim)	0.78
Laboratory B 5-day feeding 7-day period		4.0	0.52	29 1 (dry skim)	0.85
				29 1 (reconstituted skim)	0.69
Laboratory C 8-day feeding 10-day period	(Trial 1)	2.85	0.10	20 0[3]	0 60
		2.85	0.28	20.0	0.70
	(Trial 2)	4.28	0.35	30 0	1.03
		4.28	0 28	30 0	0 73
	(Trial 3)	5.70	0.68	40 0	1.20
		5.70	0.50	40.0	1 00
	(Trial 4)	5.70	0.77	40.0	1.50
	(Trial 5) 40 cc. of reconstituted non-vitamin D milk was fed without reference oil				0.06[4]

[1] The quantity of reference oil fed with the milk is the same as that used when the reference oil was fed alone
[2] The value 1 0 means a narrow continuous line of calcification.
[3] Reconstituted non-vitamin D whole milk powder used by Laboratory C.
[4] Average of 25 animals.

To obtain a satisfactory line test, assayers report the use of 28.4–65 mg. of reference oil as the total for the assay period. Thirteen use 45 mg. or less. Some report a variation in the quantity in supplement required with the season of the year.

Rickets Resistance.—Seven assayers reported rickets resistance of various degrees of severity.

Feeding of Non-Vitamin D Skim or Whole Milk with the Reference Oil.—
One assayer reported the feeding of skim milk and another the use of
fresh, whole non-vitamin D milk with the reference oil.

The most important problem is that of finding a satisfactory reference
substance other than cod liver oil or to ascertain whether the feeding of
non-vitamin D skim or whole milk with the reference oil is a more satis-
factory procedure than the use of the reference oil alone.

Table 1 shows the results obtained by three collaborators when various
levels of reference oil were compared with the same quantity of reference
oil plus the quantity of skim milk, or non-vitamin D whole milk, equal
to the volume of irradiated milk (135 U. S. P. units per quart), which
would have been fed in a routine assay. Groups of ten or more animals
were used for each supplement in all of the nine trials. Trials 1, 2, and 3
of Laboratory C are in duplicate, so that for each trial two groups of ten
animals each were fed the oil and two the oil plus milk during the same
period of time. The radii were split, stained, photographed, and scored
in the laboratory of the Associate Referee. A greater line test response
was obtained when the milk was fed with the reference oil than when the
reference oil alone was fed, and there was a greater response with non-
vitamin D whole milk than with skim milk. However, only two compari-
sons were made with skim milk, whereas seven were made with the whole
milk, and therefore more trials with skim milk will be necessary to
determine definitely the relative effect of these two types of milk. In an
earlier report, *This Journal*, **19**, 248, two of three trials showed enhance-
ment of the line response when skim milk was fed with the reference oil,
and for a third the responses of the two groups were essentially alike.
Three assays were made on the reconstituted whole milk powder with
25 animals. All the animals gave a negative response except four, and
these showed only a very slight degree of calcification. In view of the
recent report by Morgareidge and O'Brien,[1] it will be necessary to give
attention to the manner in which the oil and milk are administered, that
is, as separate supplements or as mixed supplements.

RECOMMENDATIONS[2]

It is recommended that further studies be made of the feeding of skim
milk or whole, non-vitamin D milk with the reference oil, in order to
determine whether the reference oil and a quantity of milk, equal to that
of the vitamin D milk being assayed, should be used as a reference stand-
ard instead of the reference oil alone.

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[1] *J. Nutrition*, **16**, 395 (1938).
[2] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 55 (1939).

REPORT ON PLANTS

By E. J. MILLER (Michigan State College of Agriculture
and Applied Science, East Lansing, Mich.), *Referee*

Less Common Metals.—From data obtained with a modification of the method for the determination of iodine in plant material, it is concluded that large samples of plant material can be completely burned and the iodine determined more satisfactorily and accurately than has been possible with apparatus previously used. Because of this and the fact that at least two other laboratories will be equipped with the apparatus, it is recommended that collaborative work be done on the method during the coming year.

Total Chlorine.—Collaborative work has been under way during the past year, and the associate referee will recommend the method for adoption as official, first action.

Carbohydrates.—The Referee approves the recommendations of the associate referee.

Inulin.—No results have been accomplished on the inulin problem but work is being initiated on the chemistry of timothy which will involve the determination of fructosans, and study will be extended to include fructosans in general.

Forms of Nitrogen.—No new analytical methods of significance in this field have been reported.

Hydrocyanic Acid in Plants.—No report will be presented. Because of pressure of other work, it was not possible for the associate referee to undertake the collaborative work intended. It is hoped that this work can be done during the coming year.

Sodium and Potassium.—No formal report will be presented, although some progress was made during the past year. The results with the Hicks method for potassium have been satisfactory and collaborative work is planned for next year. The recommendations of last year should stand for next year.

REPORT ON LESS COMMON ELEMENTS

MODIFICATION OF THE TENTATIVE METHOD FOR DETERMINATION OF IODINE IN PLANT MATERIAL

By J. S. McHARGUE, *Associate Referee*, and E. B. OFFUTT
(Department of Chemistry, Kentucky Agricultural
Experiment Station, Lexington, Ky.)

During the past year an effort was made in the Department of Chemistry of the Kentucky Agricultural Experiment Station to improve the combustion method that has been used for the determination of iodine

in forage crops and vegetables. It was described at a previous meeting of this Association, *This Journal*, 16, 207; *Methods of Analysis*, A.O.A.C., 1935, 8.
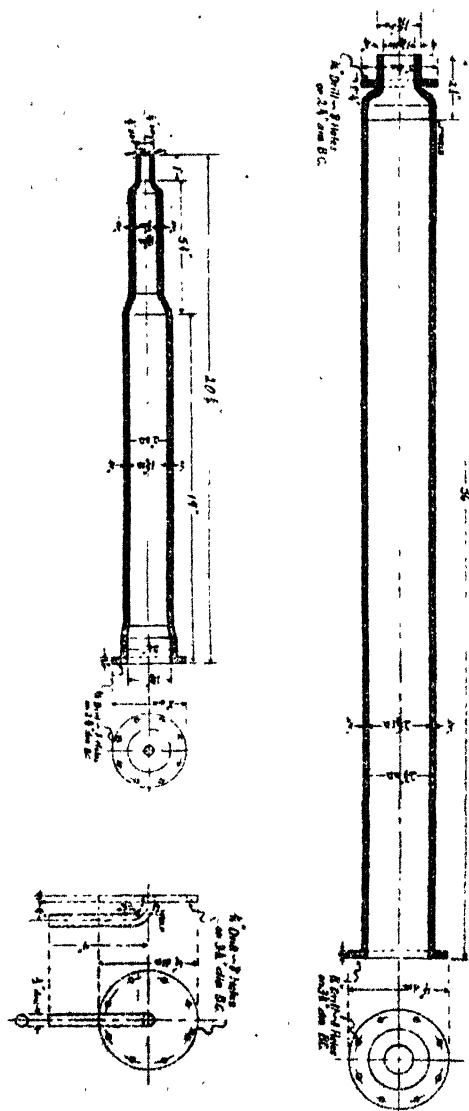
FIG. 1.—COMBUSTION TUBES.

The principal difficulties encountered with the method include: (1) maintaining an air-tight system in the silica tube during the combustion;

(2) the time required to make a complete extraction of the iodine from the potassium carbonate residue with alcohol; and (3) the complete destruction of all traces of organic matter in an electric furnace at 400° C. without loss of any iodine.

CHANGES PROPOSED IN THE METHOD

Figures 1 and 2 show the modifications that have been made in the combustion tubes and the absorption apparatus. The two sections of the combustion tube are made of Misco-B metal, which is an alloy of iron (64 per cent), chromium (24 per cent), and nickel (12 per cent), respectively. This alloy will withstand temperatures up to 1000° C. without any
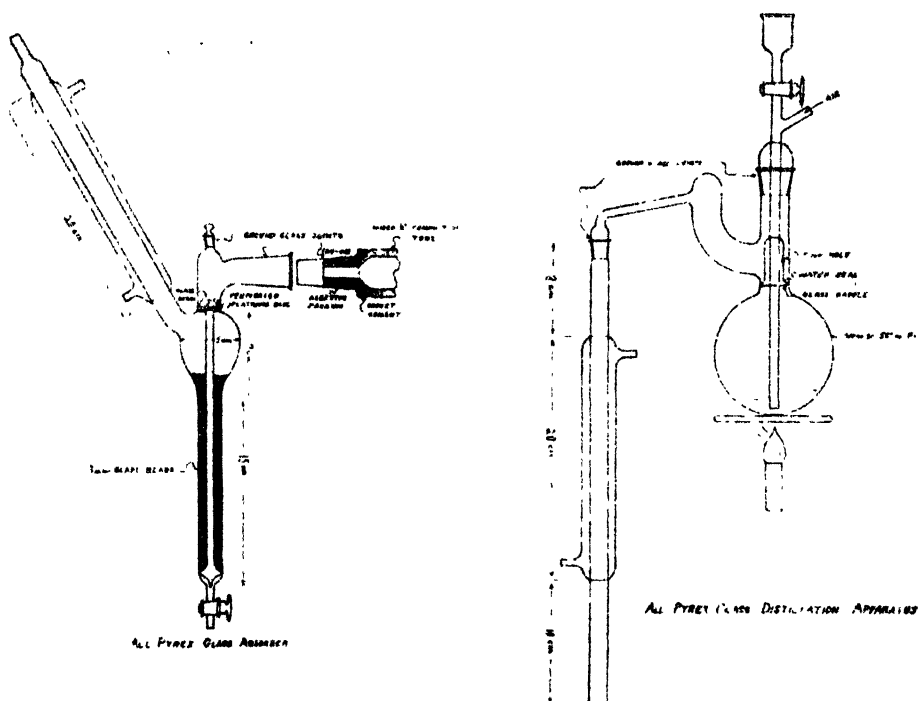
FIG. 2.—ABSORPTION APPARATUS.

serious oxidation, corrosion, or deterioration in the physical structure of the alloy. However, for the combustion of plant material it is not desirable to maintain a temperature above 500°–600° C. in the tubes. Misco-B metal is manufactured by the Michigan Steel Casting Company, Detroit, Mich., the same company that made the combustion tube according to the specifications submitted by this laboratory. The two sections of the combustion tube and the cap at the right end of the larger tube are fast-

ened together and made air-tight by means of threaded bolts and nuts and asbestos gaskets between the joints.

The new absorber was designed and constructed from Pyrex glass tubing in this laboratory by the junior author. It is connected to the combustion tube by means of a ground joint, and one section of the joint is cemented to the combustion tube with gasket cement. By means of the new absorber the volume of the absorbing solution is held at the minimum.

A U-shaped boat about 30 inches long and made of sheet nickel replaces the alundum boats previously used. It has a capacity of as much as 100 grams of finely ground, dry plant material. The sample is spread on curved pieces of nichrome wire gauze placed in the boat. This arrangement allows a current of air to flow beneath and above the sample during combustion. The short section of the combustion tube is partly filled with 20-mesh copper wire gauze, which aids in the complete combustion of any smoke that is not burned in the larger section of the combustion tube. It requires 1–3 hours to make a complete combustion, depending upon the size and the nature of the plant material burned. Leguminous plants and tobacco burn more readily than do grasses and cereals. In case of the latter materials, finely pulverized copper oxide mixed with the sample aids in the combustion. The furnaces remain the same.

After the combustion is completed the absorbing solution is transferred to a modified 300 cc. Claissen flask having ground-glass stoppers and connections. The residue of ash is also washed into the same flask. From this point the method of Stimmel and McCullagh is followed.[1]

The contents of the flask are acidified with sulfuric acid (1+1), about 5 drops of a 10 per cent solution of ferric sulfate are added, and the contents of the flask are heated to boiling and distilled. During the distillation three 2 cc. portions of a 3 per cent hydrogen peroxide solution are added at intervals through a dropping funnel into the distillation flask. The distillate is received in a 50 cc. extraction flask, which contains 0.2 cc. of a 3 per cent solution of sulfuric acid and 0.2 cc. of a 10 per cent solution of sodium sulfite. The distillation is continued until the volume in the distilling flask is reduced to a few cc. The distillation flask is disconnected from the condenser, and the condenser tube is rinsed into the receiver with distilled water. The distillate is boiled gently for about 2 minutes to expel carbon dioxide and sulfur dioxide. The solution is made slightly alkaline with 10 per cent potassium hydroxide and concentrated by boiling until the volume is about 10 cc. The solution is then made acid with 3 per cent sulfuric acid; two drops in excess of the acid and 5–10 drops of a saturated solution of bromine water are added; and the mixture is boiled gently until all the bromine is expelled. At this point in the procedure the iodine is in the form of iodate. The contents of the flask

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[1] *J. Biol. Chem.*, 116, 21 (1936).

are cooled as quickly as possible to about 15° C., two drops of a 1 per cent solution of potassium iodide and four drops of a 0.25 per cent starch solution are added, and the iodine is titrated with a 0.01 N sodium thiosulfate solution. The iodine is calculated in p.p.b.

The modified procedure was tested on a sample of kelp that contained a known amount of iodine (0.127 per cent) as determined with fusion, and colorimetrically in carbon disulfide with the use of a micro colorimeter.

Three portions consisting of 0.2 gram each of finely ground kelp were weighed into a nickel dish and fused with potassium hydroxide from 100°–400° C. until all the organic matter was destroyed. The residue was cooled, dissolved in water, and transferred to a Claissen flask, and the iodine was determined by the distillation and titration method described above. The following results for iodine were obtained on a sample of 0.2 gram of kelp.

	per cent
Iodine, colorimeter No. 1	0.127
Iodine, distillation No. 2	0.125
Iodine, distillation No. 3	0.129
Iodine, distillation No. 4	0.126
Average of 3 distillations	0.1266

For experiment 2, two 0.25 gram portions of the finely ground kelp were weighed and mixed with 25 grams of wheat straw, in which it was not possible to detect iodine. The two portions were burned separately in the modified combustion tube, and the iodine was determined according to the procedure described in this report. The following results were obtained on kelp, wheat, and tobacco.

		gram	Iodine per cent
Kelp	a	0.2500	0.1255
Kelp	b	0.2500	0.1260
			p.p.m.
Wheat grain	a	50	217
Wheat grain		75	220
Wheat grain	b	50	306
Wheat grain		100	366
Tobacco leaf	a	50	425
Tobacco leaf		50	530
Tobacco leaf	b	25	928
Tobacco leaf		25	980
Tobacco leaf	c	50	1,154
Tobacco leaf		50	1,052

From the results given and additional data not included in this report it was concluded that large samples of plant material could be completely burned and the iodine determined more satisfactorily and accurately

than was possible with the apparatus previously used. Since two other laboratories have recently submitted orders for combustion tubes made of Misco alloy metal, it is the recommendation[1] of the Associate Referee that cooperative work be sought on this subject during the next year.

REPORT ON TOTAL CHLORINE IN PLANTS

By Herbert L. Wilkins (Bureau of Plant Industry,
Beltsville, Md.), *Associate Referee*

This year's work consists entirely of collaborative studies on the tentative method presented by the Associate Referee, *Methods of Analysis, A.O.A.C.*, 1935, 131. Of the several chemists who were invited, four agreed to undertake the work.

Because the Associate Referee is recommending that the method be adopted as official, first action, and that the work be discontinued, a review of much of the material found in previous reports, *This Journal*, 17, 268; 18, 379; 19, 72, 366; 20, 335; 21, 353, is included in this one.

Unfortunately the changes recommended in the 1937 report were not included in the published version. These had to do with the preparation of the iodine solution and the change in the weight of potassium iodide required to make the standard solution of this reagent. The instructions to the collaborators included all the changes that had been made in the procedure since it was published as a tentative method, *Ibid.*, 22, 72.

The sample used was a portion of the one prepared for the 1935 report.

The collaborators who reported were T. A. Pickett of the Georgia Experiment Station, Experiment, Ga., and J. H. Mitchell and a class of his students at Clemson Agricultural College, Clemson, S. C. The Associate Referee takes this opportunity to congratulate these students on the excellence of their work, and to extend his thanks to all the collaborators for their help.

In Table 1 there is assembled all the available data on this sample. The grand average is 6.26 mg./gram of grass. The mean deviation of the 42 determinations from the grand average is ±0.09 mg. Nineteen of these analyses were made by the nine students. The average of their analyses is 6.29 mg./gram, and their mean deviation (from 6.26) is ±0.13 mg. The average of the 23 analyses by the four graduate chemists is 6.23 mg./gram and their mean deviation from 6.26 is ±0.06 mg. In terms of the chlorine content of the sample these averages would be 0.629 per cent for the students and 0.623 per cent for the chemists.

The late O. B. Winter, formerly General Referee on Plants, prepared two samples on which both he and the Associate Referee made analyses

by the tentative method. The average difference in their reports in six cases is ±0.06 mg./gram.

If all the collaborative results from the fifty-seven analyses made by 14 people are considered, the deviations of the individual values from the appropriate means average ±0.08; they have been as large as

TABLE 1.—*Chlorine found by various collaborators in a sample of dried grass*

	mg./gram		mg./gram
Associate Referee	6.26	(Prof. Mitchell's class)	
	6.26		
Own average, 6.25 mg.	6.26	T. R. Bainbridge	6.15
Deviation, ±0.01 mg.	6.27		6 00
	6.27	T. J. Blalock	
	6.24		6.18
	6.26		6.19
	6.22		
	6.25	S. J. Boyd	6.66
	6.25		6.67
O. B. Winter	6.31	L. T. Garick	6.35
	6.32		6.30
Own average, 6.34 mg.	6.30		
Deviation, ±0.03 mg.	6.35	E. W. Griffin	6.14
	6 40		6.15
J. H. Mitchell	6 07	F. L. Moore	6 18
	6.08		6.35
Own average, 6.10 mg.	6.08		
Deviation, ±0.04 mg.	6.18	M M. Nichols	6.30
			6.35
			6.40
T. A. Pickett	6.15		
	6.18	N. P. Page	6.20
Own average, 6.18 mg.	6.18		6.25
Deviation, ±0.01 mg.	6.20		
		H. A. Raysor	6.36
			6.35
Chemists' average	6.23	Students' average	6.29
Chemists' average deviation from general mean	±0.06	Students' average deviation from general mean	±0.13

Grand average 6.26 mg.
Average deviation from grand average (6.26) ±0.09 mg.

±0.41 mg. only once and have exceeded ±0.14 mg. only six times. Among the data obtained by the tentative method there are 39 analyses, of which each collaborator made at least four. When each collaborator's analyses are compared with his own mean, and the 39 resulting deviations are

averaged, ±0.02 mg. is shown as the over-all precision of the method itself.

Winter applied both the tentative and official methods to his dried beets and to the dried grass, and reported averages of 0.057 per cent (tentative) and 0.063 per cent (official) on the dried beets, and of 0.634 per cent (tentative) and 0.622 per cent (official) on the dried grass. Similarly Pickett reported for the grass averages of 0.618 per cent (tentative) and 0.636 per cent (official). The average difference shown by the three comparisons of the two methods is ±0.012 per cent or ±0.12 mg./gram.

In 1936, Winter wrote that he washed the filter paper thoroughly and obtained a faint blue color with starch on adding the iodine without adding any potassium iodide. Since he followed the Associate Referee's directions for filtering without difficulty, he did not try the Gooch as he had suggested in a previous letter. He was surprised to find the method so simple, rapid, and accurate.

Mitchell wrote that for the first determinations by his group of students he considers the results to be good. They may be too high or too low, but are quite consistent. He states that rather than have all nine students make the solutions separately, the permanganate, iodine indicator, and starch solutions were made as stock solutions (just one of each). The potassium iodide solutions were standardized by each student, and Mitchell set up three burets on a side desk for them to use. These were not calibrated burets. The time required was within two 3-hour periods. It is true that there are a great many reagents, but when once they are made the actual method is not long. There seems to be some doubt as to how much permanganate to use. If too much is used, the results will be high. Apparently the method is giving consistent results.

After obtaining low results earlier when using a different filtering arrangement and possibly also too little permanganate, Pickett wrote that he had received the duplicate samples of grass and had run several more analyses. He believes his low results are due to the fact that he did not add enough potassium permanganate. He ran a series of determinations, using exactly the same procedure except for the amount of potassium permanganate added. Dividing the analyses into three groups, he obtained 5.83, 5.87, and 5.87 mg./gram for the lower amount of potassium permanganate added; for the intermediate amount he obtained 5.95, 6.05, and 6.05 mg./gram; and for the highest amount added (which faded quite slowly) he obtained 6.15, 6.18, 6.18, and 6.20 mg./gram. He noticed that when he intentionally had an excessive amount of potassium permanganate in the blank, he obtained high results, 0.4–0.5 cc. He suggests that in the procedure more emphasis be put upon the direction "until the color fades slowly."

SUMMARY

The method presented is essentially a procedure for the determination of silver in a solution of sulfuric acid, which may vary from 3 to 40 cc. of

the concentrated acid in each 100 cc. of solution. Because it is a method for determining silver in such strongly acid solutions, it would seem to be useful whenever silver or a silver precipitable halogen can be used as a measure of the constituent sought.

The method has been shown to have a precision of ±0.02 mg. when each analyst's results are compared with his own mean, and one of ±0.09 mg. when 42 analyses by 14 analysts are compared with the general mean, including the work of a class of nine undergraduate students. The precision of the titration step in the procedure is shown by the results obtained when 10 successive equal aliquots of a solution of silver nitrate were titrated. In this experiment the average deviation from the mean (±0.015 mg.) was ±0.014 cc.

The accuracy of the method is independent of the amount of chlorine found in the range studied; it averaged ±0.03 mg. in the hands of the Associate Referee when using samples that contained 8, 13, 42, and 70 mg. of chlorine derived from a standard solution of hydrochloric acid prepared from the constant boiling acid. When average results by the tentative method were compared with results from the official or a modified official method, a value of ±0.12 mg. was obtained as the average of three such comparisons.

The method is applicable to a wide variety of substances because the modified open Carius digestion, which is used to disintegrate the sample, will decompose many organic materials and because the silver chloride and sample residue separated from the nitric acid digest are completely decomposed by the Kjeldahl digestion, thus providing a solution that is free of organic matter and of halides in which to titrate the resulting silver sulfate with potassium iodide.

The method has demonstrated its suitability for the use of the general technician by the success it gave in the hands of the collaborators, especially those of undergraduate standing. It requires no excessive amount of time as evidenced by the comments of the collaborators and by the fact that the students standardized their potassium iodide solutions and completed their analyses in two 3-hour laboratory periods.

It is not known to what extent the accuracy or precision of the method may be increased by the introduction of the elaborate precautions common to the atomic weight type of investigation, but such measures applied to this procedure should repay any worker who has need for such accuracy and precision. The method appears to be suitable for micro analysis because the end point is still very sharp when both the standard solution concentration and the titration volume are reduced to one-tenth of those given in the procedure.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method discussed be adopted as official (first action).

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 54 (1939).

(2) That a statement of the limitations of the applicability of the present official (alkaline ignition) method be made a part of that method if it is retained.

(3) That the appointment of an Associate Referee on Total Chlorine in Plants be discontinued.

REPORT ON CARBOHYDRATES IN PLANTS

By J. T. SULLIVAN (Division of Forage Crops and Diseases, Bureau of Plant Industry, in cooperation with the Northeastern States, State College, Pa.), *Associate Referee*

The last report of the Associate Referee, *This Journal,* **19,** 371, contained a recommendation for an improved procedure for the titration of reduced copper. The present report offers no new information but contains a brief discussion of the status of the methods and recommendations for new work.

REDUCING SUGARS

The reducing sugar methods that have been approved as tentative or official (first action) are the Munson and Walker and the Quisumbing and Thomas procedures, and for the determination of the reduced copper the gravimetric, the electrolytic, and the permanganate titration methods. Many other methods are in use, some having distinct manipulative advantages. Despite the need for standardization, the Associate Referee hesitates to place emphasis upon any of these other methods until more information about them is available. It has been pointed out a number of times that results by several methods vary with different plants. All oxidizing reagents are more or less susceptible to reaction with non-sugar reducing substances. Methods in which the excess cupric copper is determined after the reduction or in which the cuprous copper is determined without removing it from the reduction mixture are particular offenders in this respect, since an iodometric titration is usually involved. The potassium ferricyanide methods are subject to the same error. Some error is involved in the actual reduction but even more is due to the reactivity of the iodine to non-sugar substances. Those methods that do not involve an iodine titration in the presence of the reduction mixture should be studied further. At the present time no method can be recommended for indiscriminate use on all plant materials.

SUCROSE

The cold acid hydrolysis method and the invertase method are tentative. In the latter case no definite procedure has been recommended. Neither method is specific for sucrose, but the invertase method gives a

definite end point of hydrolysis with some plant materials but not with others, as shown in a former report, *This Journal*, 16, 471.

STARCH

The malt diastase method with subsequent acid hydrolysis is tentative. Takadiastase is also in wide use. Recently published methods are directed away from diastatic reagents and toward procedures that are more specific for starch; they involve solution and reprecipitation of the starch or of its iodine complex and often hydrolysis of a more or less purified starch. Such methods have shown that the vegetative parts of plants, especially the leaves, contain less starch than was previously supposed. These methods should be studied.

NEW STUDIES

It is recognized that many plants contain, in the absence of or in addition to starch, levulosans or fructosans, which on hydrolysis yield fructose. They are important physiologically and have a function similar to starch as carbohydrate reserves. Their analysis involves new problems of hydrolysis since fructose is less stable than glucose to acid-hydrolyzing reagents.

Since it has been shown that fructosans may yield a certain amount of glucose in addition to levulose, methods should be studied for the determination of glucose and fructose in mixtures. It is also of interest to determine them when they occur together and free in plants. The many different types of methods that have been proposed should be studied, especially Erb and Zerban's[1] recent combination of the Jackson and Mathews[2] method for fructose with the Munson and Walker method for reducing sugars.

RECOMMENDATIONS[3]

It is recommended—

(1) That methods for the determination of reducing sugars, sucrose, and starch be further studied,

(2) That studies be begun on methods for the determination of fructosans in plants,

(3) That studies be begun on methods for the determination of glucose and fructose in plants.

No report on inulin was given by the associate referee.

No report on forms of nitrogen was given by the associate referee.

No report on hydrocyanic acid was given by the associate referee.

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[1] *Ind. Eng. Chem. Anal. Ed.*, 10, 246 (1938).
[2] *Bur. Standards J. Research*, 8, 403 (1932).
[3] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 64 (1939)!

No report on sodium and potassium was given by the associate referee.

No report on lignin was given by the referee.

No report on enzymes was given by the referee.

No report on papain was given by the associate referee.

REPORT ON WATERS, BRINE, AND SALT

FLUORINE IN WATER

By ANNA E. MIX (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

An artificial mineral water solution was prepared for collaborative work; it contained 2193 p.p.m. of chlorides, 1465 p.p.m. of sulfates, 28 p.p.m. of phosphates, and 2.12 p.p.m. of fluorine.

The thorium nitrate titration method was suggested in view of the results obtained last year. The following collaborators reported:

COLLABORATORS

R. W. Bridges, Aluminum Co. of America
P. J. Buchanan, American Agricultural Chemical Co.
J. N. Carothers, Monsanto Chemical Co.
J. R. Davies, General Foods Corporation
W. K. Enos, Virginia-Carolina Chemical Corporation
J. B. Fullerton, The Upjohn Company
M. Given, Crystal Gelatine Co.
V. L. Harnack, United Chemical and Organic Products
H. B. Hodge, Lucidol Corp.
Simon Klosky, American Agricultural Chemical Company
H. V. Moss, Monsanto Chemical Company
W. C. Motz, Virginia Chemical Corporation
K. B. Peterson, North Dakota Regulatory Dept.
W. B. Sherry, General Chemical Company
W. E. Stokes, Standard Brands
O. I. Struve, Eastern States Cooperative Milling Corporation
J. J. Vollertson, Armour and Company

Because the collaborative results show that many of the analysts used variations of the suggested A.O.A.C. method the results are listed in two tables. Table 1 shows results obtained by the analysts that adhered strictly to the A.O.A.C. method, and Table 2 shows the results of those that deviated from the A.O.A.C. method.

The results of both tables were studied by the technic of analysis of variance.[1] The first findings are (1) that either by strict adherence to the

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[1] Snedecor, Geo. W., Statistical Methods. Collegiate Press, Inc., Ames, Iowa.

TABLE 1.—*Results obtained by A.O.A.C. method*
(Fluorine present, 2.12 p.p.m.)

COLLABORATOR	NO. OF TRIALS	ACIDITY 0.02 N HCl	FLUORINE FOUND	
		aliquot	*p.p.m.*	
1	3	0.2 cc./25 cc.	2.0	
			2.0	Av. 1.97
			1.9	
2	2	0.95 cc./40 cc.	2.44	Av. 2 42
			2.40	
3	3	0.35 cc./40 cc.	1.35	
		0.20 cc./40 cc.	1.35	Av. 1.53
			1.90	
4	6	0.3 cc./40 cc.	2.3	
		0 3 cc./40 cc.	2.4	
		0.2 cc./40 cc.	2.4	
		0.2 cc./40 cc.	2 4	Av. 2.25
		0.2 cc./40 cc.	2.0	
		0.2 cc./40 cc.	2.0	
5	1	—	1.80	
6	2	0.2 cc./40 cc.	1.50	Av. 1.50
		0 2 cc./40 cc.	1.50	
7	7	1 05 cc./40 cc.	1 8	
		0.45 cc./40 cc.	1.9	
			1.8	
			2.1	Av. 2.0
			2.0	
			2.1	
			2.3	
8	8		1.87	
			1.80	
			1.82	
			1.80	Av. 1.88
			1.77	
			1.80	
			2.10	
			2.07	
	32		62.67	

Average recovery, 1.96 p.p.m.
Standard deviation, $\sigma_1 = 0.2871$.

given procedure, or by modifications of it, individual analysts can secure closer replication of measurements than agreement with other analysts, and (2) that a comparison of the variances within means for the two sets, arising from the errors of replication by the individual analysts, shows

TABLE 2.—*Results obtained by various modifications of A.O.A.C. method*
(Fluorine present, 2.12 p.p.m.)

COLLABORATOR	NO. OF TRIALS	FLUORINE FOUND (CALCULATED TO WEIGHTED AVERAGES)		VARIATION USED
		p.p.m.		
1	4	1.3 1.3 1.0 1.35	Av. 1.24	Own method of titrating
2	4	1.8 1.7 1.75 1.75	Av. 1.75	Not same number of cc. Th(NO$_3$)$_4$ in standard as in sample soln. Acidity, 2.09; in standard, 2.09
3	2	1.82 1.75	Av. 1.79	Acidity, 4.80—high
4	4	2.1 1.8 2.4 1.6	Av. 1.98	Acidity, 24.4, 6.5, 24.0 Own method of titration
5	6	1.4 4.0 1.4 1.4 1.9 1.2	Av. 1.89	No evaporation; H$_3$PO$_4$ instead of HClO$_4$ No constant aliquot
6	1	3.26		No evaporation; H$_3$PO$_4$ instead of HClO$_4$
7	1	2.50		Control of acidity before titration different
8	2	2.2 2.1	Av. 2.15	Own method of acidity control
9	2	0.8 0.8	Av. 0.80	Acidity, 3 cc. HCl
10	2	1.65 1.75	Av. 1.70	Acidity, 3.4 cc. Added 1.7 instead of 2 cc. to standard soln.
11	4	2.39 2.41 2.45 2.41	Av. 2.42	Acidity much too high
	32	59.46	Av. $\dfrac{59.46}{32}$ = 1.86	

Standard Deviation, $\sigma_2 = .6855$

$$\frac{\sigma_2}{\sigma_1} = \frac{.6855}{.2871} = 2.39$$

that any one analyst, by strict adherence to the A.O.A.C. method, obtains a greater consistency in results than by favoring the particular modifications attempted in this study.

TABLE 3.—*Analysis of variance—collaborative study*

SOURCE OF SUM OF SQUARES	SUM OF SQUARES	DEGREES OF FREEDOM	VARIANCES	F
A.O.A.C. Method — mean value = 1.96 p.p.m.				
Total	2.5104	31		
Among Means	1.9867	7	.28381	9.208
Within Means	0.6237	24	.025988	
Modifications of A.O.A.C. method — Mean = 1.86 p.p.m.				
Total	15.0517	31		
Among Means	7.7351	10	.77351	2.22
Within Means	7.3166	21	.34841	

Further inspection suggests that the mean result of the collaborators following strictly the A.O.A.C. method (1.96 p.p.m.) is obviously low. Two causes for this are suggested: (1) The departure may be one of chance alone, arising from differences in operation by individual collaborators having slightly different interpretations of the wording of the method; (2) waters high in chlorides may not be amenable to the silver perchlorate treatment to control acidity. The method may be expected to produce more accurate results when used on samples containing moderate amounts of chlorides.

A survey of Table 2 indicates the manner in which the non-A.O.A.C. group departed from the standard procedure. Since these departures are accompanied by deviations in fluoride recoveries far too great to be explained by accident of errors of replication, a few general precautions seem to be in order.

1. Perchloric acid must be used for the distillation. Phosphoric acid is unsuitable.

2. The aliquot of the sample, made alkaline to phenolphthalein, must be evaporated to 20 cc. or less before the fluorine is liberated as hydrofluoric acid. Real importance is attached to this simple step.

3. Collaborative results indicate that the acidity of the solution for titration should contain 2 ml. of free hydrochloric acid (1+249) instead of some other amount. The standard and sample tubes must contain equal acid concentrations.

4. To maintain validity of color comparisons between sample and standard tubes, both must contain the same quantity of thorium nitrate, in the same volumes of liquid.

It is concluded from this study that the A.O.A.C. method for the

determination of fluorine, while in need of clarification, gives more accurate, more consistent, and more reproducible results than do the variations here attempted.

No report on effervescent salts was given by the associate referee.

No report on dairy products was given by the referee.

No report on butter—preparation of sample and fat—was given by the associate referee.

REPORT ON CHEESE

By IRA D. GARARD (New Jersey College for Women,
Rutgers University, New Brunswick, N. J.),
Associate Referee

The only project undertaken this year was a study of methods for the extraction of fat for examination. In addition to the two official methods, a third method, which has been used with some types of cheese,[1] was also given some consideration. This last method consists of melting the cheese at 45° to 50° C. on the wall of a beaker and allowing the fat to drain to the bottom of the beaker. To date the investigation has not advanced beyond the preliminary stages.

It is recommended[2] that a further study be made of the applicability and relative advantages of the different methods available for the extraction of fat from cheese.

REPORT ON MALTED MILK

By FRED HILLIG (U. S. Food and Drug Administration,
Washington, D. C.), *Associate Referee*

Last year the unified method for determination of fat proposed by the Associate Referee, *This Journal*, **18**, 454, was submitted to collaborative tests. Certain precautions, as outlined in the report last year, were incorporated in the text of the method. The results obtained by five collaborators were not entirely satisfactory.

This year a chocolate flavored malted milk, prepared in the laboratory, was sent to collaborators with the request that the fat be determined by the method used last year. The results are given in Table 1.

The results are entirely satisfactory. Good correlation, both by individual analysts and between analysts, was obtained.

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[1] Garard, Minsky, Baker, and Pascale, *Ind. Eng. Chem.*, **29**, 1167 (1937).
[2] For report of Subcommittee C and action by the Association, see *This Journal*, **22**, 60 (1939).

TABLE 1.—*Collaborative results on chocolate flavored malted milk*

ANALYST	FAT
	per cent
1	6.75
	6.72
2	6.85
	6.83
	6.84
3	6.74
4	6.84
	6.85
	6 90

Results of a collaborative study on two samples of malted milk submitted to eleven collaborators who used the tentative method are given in the report of the Associate Referee in 1936, *This Journal*, 19, 382. They are unsatisfactory. In this report attention is called to three factors: (1) the small quantity of sample taken, (2) the occurrence of emulsions, and (3) difficulty in removal of non-fat material from the dried fat, which may be the cause of the poor agreement obtained by the collaborators.

The method for the determination of fat in dried milk is official. This method calls for the use of ammonia in preparing the sample for extraction. The procedure has become recognized as standard practice in analyzing for fat in milk products. The tentative method for fat in malted milk does not call for the use of ammonia, but otherwise it is similar to the method for dried milk. It is, therefore, recommended that a study be made on the application of this method to the determination of fat in malted milk. The unified method should also be compared to the tentative method for fat in malted milk and to the method for fat in dried milk.

Since the unified method is suitable for obtaining sufficient fat for the Reichert-Meissl determination, it is believed that it should be further studied with a view to its adoption for this purpose. It should be borne in mind also that the unified method is suitable for the determination of fat in food products other than milk products and also furnishes a convenient means for obtaining sufficient fat from most milk products for the determination of the Reichert-Meissel number.

Appreciation is expressed to Doris H. Tilden, Food and Drug Administration, San Francisco, and to Marie L. Offutt and J. H. Loughrey, Food and Drug Administration, New York, for their splendid cooperation.

RECOMMENDATIONS[1]

It is recommended—

(1) That the Associate Referee study the application to malted milk,

of the official method for the determination of fat in dried milk, with a view to final adoption of a single method for both products.

(2) That methods for the separation of fat from dairy products, except cheese, for the determination of fat constants be further studied.

(3) That studies of methods for the determination of casein be continued.

REPORT ON MALTED MILK—MICRO-ANALYTICAL METHODS

By B. G. HARTMANN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Several years ago the Association recommended that the glycerol-alcohol-water mixture (equal volumes) proposed by C. W. Ballard, New York Department of Health, be tried as a mounting medium in the microscopical examination of malted milk.

Under the supervision of G. L. Keenan, Microanalytical Division, Department of Agriculture, Washington, D. C., a number of commercial samples containing malted milk in varying amounts were examined, the mixture and mineral oil being used.

In all cases there was a pronounced tendency toward disintegration in the Ballard mixture, whereas the mineral oil left the material in suspension, so that the stippled appearance of malted milk remained plainly discernible.

While the Ballard mixture may be preferable to mineral oil in cases where clearing of certain ingredients is desired, its use in the identification of malted milk ingredients, particularly those that are soluble in aqueous vehicles, can not be recommended.

It is recommended that the study of the Ballard mixture be discontinued.

At the last meeting of the Association further collaborative study of the tentative method for the determination of citric acid in milk was recommended.

In this year's report on fruits and fruit products the Referee points out that the slow oxidation of a brominated citric acid solution yields materially more pentabromacetone than does the rapid oxidation procedure prescribed in the tentative method. Therefore, an investigation of this phase of the determination is recommended.[1]

Pending the outcome of the investigation it is recommended that further study of the present method for the determination of citric acid in milk be deferred.

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[1] For report of Subcommittee C and action by the Associatio , see *This Journal*, 22, 61 (1939).

REPORT ON DRIED MILK

By Fred Hillig (U. S. Food and Drug Administration,
Washington, D. C.), *Associate Referee*

It was recommended last year that methods for the determination of lactic acid in dried milk be further studied. Accordingly, two samples of dried skim milk were submitted to collaborators with the request that lactic acid be determined by the colorimetric and the Troy and Sharpe aldehyde methods. The results are given in the table under Report on Neutralizers in Dairy Products (p. 495) and will not be repeated here. It is recommended that the work be continued.

No report on milk proteins was given by the associate referee.

No report on gelatin in milk and cream was given by the associate referee.

REPORT ON LACTOSE IN MILK

EFFECT OF VOLUME OF PRECIPITATE ON ACCURACY OF POLARIMETRIC DETERMINATION*

By E. R. Garrison (Department of Dairy Husbandry,
Missouri Agricultural Experiment Station,
Columbia, Mo.), *Associate Referee*

The procedure specified by the Association of Official Agricultural Chemists for the polarimetric determination of lactose in milk, *Methods of Analysis, A.O.A.C.*, 1935, 266, is based upon the method followed by Wiley (1884, 1885).[1] Wiley used 61.68 grams of milk in his work and found that the precipitated protein occupied a volume of 2.4 ml. He accordingly diluted his milk samples to 102.4 ml., after the addition of the clarifying agent, in order to prepare 100 ml. of solution. The present A.O.A.C. method specifies 65.8 grams of milk (twice the normal weight), which is diluted to 102.6 ml. after addition of the mercuric nitrate reagent. This dilution is proportional to that made by Wiley for a slightly smaller weight of milk. Wiley does not state whether he worked with whole or with skimmed milk but he failed to make any allowance in his dilution for any fat, which if present would be carried down with the protein and contribute to the volume of precipitate. The present A.O.A.C. method likewise does not make any allowance in the dilution for any variation in the volume of the precipitate, which would obviously vary with the fat and protein content of the milk.

Wiley and Ewell (1896)[2] noted that the volume of precipitate varied

with the composition of the milk and that it was necessary to dilute the sample according to the volume of precipitate if accurate results were to be obtained. They, therefore, proposed the method of double dilution for the accurate polarimetric determination of lactose in milk. Twice the normal weight of milk is placed in a 100 ml. and in a 200 ml. flask and the clarifying agent is added; the flasks are then filled to the mark with water and shaken, after which the contents are filtered and polarized. A mathematical formula was also devised by these individuals for calculating the true polarization from the results obtained. It may be summarized as follows: divide the product of the two readings made from the solutions in the 100 ml. and 200 ml. flasks by the difference in these readings. Woodman (1915)[1] proposed this same method, but used a slightly different procedure for calculating the percentage of lactose in the milk.

Scheibe (1901)[2] emphasized that the volume of precipitate depends upon both the protein and the fat content of the milk and that a serious error results in the optical determination of lactose if the sample is not diluted in accordance with the volume of precipitate. Milk samples containing 2.8–4.7 per cent fat had an average volume of 5.8 ml. of precipitate. He adopted the procedure of diluting the milk sample to 100 ml. after addition of the clarifying reagent, but multiplied the polarization results by 0.94 in order to correct for the volume of precipitate.

Ruffy (1932)[3] compared the polarimetric and gravimetric methods of determining lactose in milk. He attributed the lack of a consistent relationship in the differences in the results secured by the two methods to an error in the optical method because of failure to allow for variations in the volume of precipitate in the different samples of milk. He used a value of 6 ml. for the volume of precipitate in his samples.

Lactose determinations made on several samples of milk by the polarimetric and the Shaffer-Somogyi (1933)[4] copper-iodometric methods, in the Dairy Department at the University of Missouri, have shown consistently higher results by the former method. It was thought that inadequate allowance for the volume of precipitate in the polarimetric method might account in part at least for the higher results secured by this method. A study was therefore undertaken to determine the variation in the volume of precipitate in different samples of milk and to compare the lactose values obtained by use of the polariscope when these samples were diluted according to the A.O.A.C. specifications and when diluted according to the volume of precipitate.

PROCEDURE

In order to secure samples with a wide range of fat and protein content, the milk from 34 individual Holstein and Jersey cows was used in this

[1] *Food Analysis.* McGraw-Hill Book Co., New York.
[2] *Z. anal. Chem.*, **40**, 1.
[3] *Lait*, **12**, 95.
[4] *J. Biol. Chem.*, **100**, 695.

study. The fat content of these samples was determined by means of the Babcock test. The fat (ml.) in the amount of milk used for the lactose determinations was computed on the basis that the specific gravity of the fat was 0.92 at 20° C. The specific gravity of the milk was determined at 20° C. with a standardized Quevenne lactometer.

The protein (ml.) in the 65.8 grams of milk used for the lactose determination was computed from the total nitrogen determinations made in duplicate on 2 ml. of milk by the Kjeldahl method. The milk was pipetted at 20° C. and the weight of milk transferred was calculated from the specific gravity. A 5 per cent deduction from the total nitrogen value was made before the nitrogen (grams) was multiplied by 6.38 for conversion into grams of protein. Rowland (1938)[1] showed that approximately 5 per cent of the total nitrogen in normal milk is non-protein nitrogen. The protein (grams) was converted into protein (ml.) on the basis that the specific gravity was 1.35 at 20° C. The fat and protein were added together to determine the total volume of precipitate in each sample.

The milk samples, precipitating reagents, and dilution water were tempered in a water bath at 20° C. before being used for the lactose determinations. Twice the normal weight of milk (65.8 grams) was weighed into each of two 100 ml. flasks and into a 200 ml. flask. The samples were clarified by the addition of 15 ml. of acid mercuric nitrate solution (1:5 dilution) and 15 ml. of a 5 per cent phosphotungstic acid solution. One of the 100 ml. flasks and the 200 ml. flask were diluted to the mark with water, while the other 100 ml. flask was diluted to 102.6 ml. The flasks were well shaken, and the contents were filtered and polarized. The percentage of lactose was determined by the method of Wiley and Ewell (1896)[2] from the polariscope readings on the solutions from the 100 and 200 ml. flasks; the polariscope readings on the solution from the 102.6 ml. flask furnished the A.O.A.C. lactose values, while the solution from the 100 ml. flask supplied the data from which was calculated the lactose value if the sample had been diluted according to the volume of precipitate in the milk.

DISCUSSION OF RESULTS

The fat and protein in the samples of milk analyzed are shown in Table 1. The fat content of the 19 samples of Holstein milk ranged from 2.9 to 4.7 per cent; the protein and fat in 65.8 grams (2 N weight) of these samples varied from 3.37 to 5.10 ml., with an average volume of 4.17 ml. The percentage of fat in the 13 samples of Jersey milk varied from 4.1 to 8.7; the protein and fat in 65.8 grams of these samples ranged from 5.01 to 8.51 ml., with an average volume of 6.65 ml. The average volume of protein and fat in twice the normal weight of milk varied with the fat content as follows:

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[1] *J. Dairy Research*, **9**, 42.
[2] *Loc. cit.*

per cent fat	ml.
2.9–3.4	3.77
3.5–4.4	4.42
4.5–5.4	5.29
5.5–6.4	6.40
6.5–8.7	7.60

TABLE 1.—*Volume of precipitate in 65.8 grams of milk from individual cows*

COW	FAT	FAT[1] IN 65.8 GRAMS MILK	PROTEIN NITROGEN[2] IN MILK	PROTEIN[3] IN 65.8 GRAMS MILK	PROTEIN[4] IN 65.8 GRAMS MILK	PROTEIN AND FAT IN 65.8 GRAMS MILK
	per cent	ml.	per cent	grams	ml.	ml.
H726	2.9	2.10	.41	1.721	1.27	3.37
H741	3.0	2.15	.45	1.889	1.40	3.35
H750	3.0	2.15	.45	1.889	1.40	3.55
H685	3.3	2.35	.43	1.805	1.34	3.69
H740	3.3	2.35	.43	1.805	1.34	3.69
H743	3.2	2.29	.50	2.099	1.55	3.84
H722	3.5	2.50	.45	1.889	1.40	3.90
H744	3.6	2.57	.47	1.973	1.46	4.03
H616	3.4	2.43	.54	2.267	1.68	4.11
H720	3.8	2.71	.45	1.889	1.40	4.11
H724	3.7	2.66	.49	2.057	1.52	4.18
H729	4.0	2.85	.45	1.889	1.40	4.25
H714	3.4	2.43	.62	2.603	1.93	4.36
H727	4.0	2.85	.50	2.099	1.55	4.40
H725	4.2	3.00	.50	2.099	1.55	4.55
H751	4.4	3.15	.50	2.099	1.55	4.70
H611	4.3	3.07	.53	2.225	1.67	4.74
H709	4.3	3.07	.55	2.309	1.71	4.78
J880	4.1	2.93	.67	2.813	2.08	5.01
H707	4.7	3.36	.56	2.351	1.74	5.10
J889	4.8	3.43	.56	2.351	1.74	5.17
J856	5.4	3.85	.57	2.393	1.77	5.62
J901	5.6	4.00	.58	2.435	1.80	5.80
J883	5.7	4.07	.68	2.855	2.12	6.19
J893	5.8	4.15	.69	2.897	2.15	6.30
J853	6.0	4.29	.74	3.107	2.30	6.59
J877	6.6	4.71	.62	2.603	1.93	6.64
J882	6.4	4.57	.69	2.897	2.15	6.72
J839	6.3	4.51	.74	3.107	2.30	6.81
J893	7.3	5.21	.57	2.393	1.78	6.99
J884	6.7	4.80	.76	3.190	2.36	7.18
J838	8.3	5.93	.73	3.064	2.27	8.20
J887	8.7	6.21	.61	2.561	1.90	8.11
J864	8.0	5.71	.90	3.778	2.80	8.51

H = Holstein.

J = Jersey.

[1] Assuming sp. gr. of fat to be 0.92 at 20°C.
[2] 5% deduction made from total nitrogen for non-protein nitrogen.
[3] Grams nitrogen×6.38.
[4] Assuming sp. gr. of protein to ● 1.35 at 20°C.

These results (Table 1) seem particularly significant when it is recalled that the A.O.A.C. method only allows for a volume of 2.6 ml. of precipi-

TABLE 2.—*Lactose values for milk samples of different composition obtained by A.O.A.C. method and when diluted according to the volume of precipitate*

| PROTEIN AND FAT IN 65.8 GRAMS MILK | LACTOSE (PER CENT) | | | DIFF. IN LACTOSE (PER CENT) BETWEEN— | |
	A.O.A.C. METHOD	DOUBLE DILUTION METHOD	DILUTED ACCORDING TO ML. PPT.[1]	A.O.A.C. AND DOUBLE DILUTION METHOD	DOUBLE DILUTION METHOD AND DILUTED ACCORDING TO ML. PPT.
ml.					
3.37	4.90	4.65	4.79	.25	−.14
3.55	5.35	5.05	5.26	.30	−.21
3.55	5.00	4.78	4.95	.22	−.17
3.69	4.90	4.73	4.85	.17	−.12
3.69	5.48	5.28	5.38	.20	−.10
3.84	5.33	5.18	5.18	.15	.00
3.90	5.05	4.85	4.96	.20	−.11
4.03	5.20	5.13	5.07	.07	+.06
4.11	4.88	4.73	4.78	.15	−.05
4.11	4.95	4.73	4.88	.22	−.15
4.18	5.38	5.13	5.28	.25	−.15
4.25	5.00	4.85	4.89	.15	−.04
4.36	4.68	4.50	4.55	.18	−.05
4.40	5.30	5.20	5.17	.10	+.03
4.55	5.13	4.85	5.02	.28	−.17
4.70	5.00	4.80	4.87	.20	−.07
4.74	4.88	4.60	4.78	.28	−.18
4.78	4.78	4.58	4.66	.20	−.08
5.01	4.90	4.60	4.76	.30	−.16
5.10	5.05	4.88	4.88	.17	.00
5.17	5.25	4.95	5.14	.30	−.19
5.62	4.30	4.20	4.17	.10	+.03
5.80	5.38	5.15	5.20	.23	−.05
6.19	4.80	4.60	4.66	.20	−.06
6.30	5.03	4.80	4.80	.23	.00
6.59	4.43	4.18	4.25	.25	−.07
6.64	4.95	4.68	4.81	.27	−.13
6.72	5.03	4.83	4.85	.20	−.02
6.81	3.90	3.63	3.77	.27	−.14
6.99	4.55	4.40	4.28	.15	+.12
7.13	4.63	4.50	4.39	.13	+.11
8.11	4.25	4.00	4.07	.25	−.07
8.11	4.83	4.65	4.53	.18	+.12
8.51	4.23	4.03	3.94	.20	+.09

[1] Calculated from value obtained when diluted to 100 ml.

tate in the dilution of the milk. Whole milk of ordinary composition contains about twice as much precipitate as the present method makes allowance for.

The values found in this study are slightly lower than those reported by Scheibe (1901)[1] for milk of similar fat content. It would seem to be more consistent to vary the allowance for the volume of precipitate with the percentage of fat in the milk (since this is easily ascertained and since protein content is closely correlated with fat content) than to adopt an arbitrary standard for all milks regardless of their composition. The proper value to allow for the volume of precipitate could be ascertained by making a compilation from analyses reported in the literature of the amount of protein in a large number of milk samples of definite fat contents. The other alternative would be to follow the method of double dilution proposed by Wiley and Ewell.

The results of the lactose determinations on the samples of milk are given in Table 2. As would be expected the A.O.A.C. method gave higher values in all cases than did the Wiley and Ewell double dilution method and the calculated values based on full allowance for all protein and fat in the sample. The A.O.A.C. values ranged from a minimum of .07 to a maximum of .30, with an average value .21 per cent higher than the values obtained by the double dilution method. The differences in the results by the two methods, however, do not show any consistent variation with the amount of fat and protein in the milk. The amount of lactose in the milk would also be a factor affecting any differences in the results by the two methods.

The double dilution method gave values that were slightly lower in most cases than the calculated results based on the volume of precipitate but with seven samples the results were higher by the latter method.

CONCLUSIONS

From the data presented it is evident—

(1) That the volume of precipitate varies with the composition of the milk and is determined by the amount of both the fat and the protein in the milk.

(2) That the present A.O.A.C. allowance of 2.6 ml. for the volume of precipitate in 65.8 grams (2 *N* weight) of milk is too low for whole milk of average composition.

(3) That the polarimetric determination of lactose in whole milk by the A.O.A.C. method averages about 0.20 per cent higher than when the milk is diluted according to the volume of precipitate so that 100 ml. of solution is obtained from 65.8 grams of milk.

It is recommended that this study be continued by the referee with other collaborators in order to secure information on which to make recommendations to the Association for any changes considered desirable for the polarimetric determination of lactose in milk.

The Associate Referee wishes to acknowledge the collaboration on this

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[1] *Loc. cit.*

study of L. D. Haigh of the Agricultural Chemistry Department, University of Missouri.

REPORT ON EXTRANEOUS MATTER IN DAIRY PRODUCTS

METHOD FOR ESTIMATION OF AMOUNT OF MOLD MYCELIA IN BUTTER

By J. D. WILDMAN (Microanalytical Division, U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

A method for estimating the amount of mold in butter was described in *This Journal*, 20, 93. The method has been used since that time in the Microanalytical Laboratory of the Food and Drug Administration, for experimental work in the Chicago Station of the Food and Drug Adminisstration, for experimental work at Purdue University, and for regulatory work by the North Dakota Regulatory Department. The method has received no adverse criticism from these sources.

Since the method is essentially the preparation of a butter sample in such a way as to render it adaptable to examination by the Howard method, *Methods of Analysis, A.O.A.C.*, 1935, 500, and since that method has been made official, it is considered that the butter method should be adopted as tentative. In this connection, as with the Howard method for tomato products, it should be borne in mind that adequate training under an experienced analyst is essential to proper use of the method. The modified method was published in *This Journal*, 22, 76 (1939).

It is recommended[1] that the method of sample preparation of butter for examination for mold by the official method for mold in tomato products be made tentative.

No report on decomposition in dairy products was given by the Associate Referee.

REPORT ON NEUTRALIZERS IN DAIRY PRODUCTS

By FRED HILLIG (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

It was recommended last year that studies of methods for the detection of neutralizers in dairy products be continued. Accordingly, two samples of dried skim milk were sent to collaborators with the request that determination of lactic acid be made by both the colorimetric

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method and the Troy and Sharpe aldehyde method.[1] It was also requested that the titratable acidities and the alkalinities of the total ash and water-soluble ash be determined. The results are given in Table 1.

TABLE 1.—*Analysis of dried skim milks*

COLLABORATOR	ACIDITY cc. 0.1 N PER 100 GRAMS		ALKALINITY OF ASH cc. 0.1 N PER 100 GRAMS		ALKALINITY OF WATER-SOLUBLE ASH cc. 0.1 N PER 100 GRAMS		LACTIC ACID			
							COLORIMETRIC METHOD PER CENT		TROY & SHARPE METHOD PER CENT	
	SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2	SAMPLE 1	SAMPLE 2
1	196	142	740	802	9.2	11.0	0.104	0.341	—	—
	200	142	736	798	9.8	11.6	0.108	0.341	—	—
2			701	785			0.039	0.278	—	—
	171	118	717	790	12.6	17.6	0.038	0.274	—	—
3	215	156	—	792	17.2	23.2	0.045	0.186	—	—
	211	157		794	17.2	25.2	0.054	0.190	—	—
4	186	130	730	794	20.0	34.0	0.150	0.350	—	—
5	202	133	692	792	12.4	25.8	—	—	0.116	0.295
	200	133	698	790	12.4	26.8			0.126	0.295
6	—	140	—	788	—	13.0	—	—	—	0.260
		140		784		12.6				0.260
7	—	128	—	790	—	22.0	—	—	—	0.272
8	—	124	—	772	—	33.0	—	—	—	0.290
		120		774		34.8				0.290

The results for the most part are disappointing. For the determination of acidity 5 grams of the milk was diluted with 100 cc. of water and titrated with 0.1 N sodium hydroxide, phenolphthalein being used as indicator. The results reported show that individual analysts agree very closely, but the checks obtained by the various collaborators are not satisfactory. During the coming year attention will be given to the Van Slyke and lead acetate methods for the determination of acidity. There are no procedures in *Methods of Analysis, A.O.A.C.*, for the determination of total alkalinity and alkalinity of water-soluble ash in milk. The collaborators were asked to make these determinations following the methods given in the wine chapter. It is very evident from the results given in the table that these methods are not applicable to milk. The Troy-Sharpe method gave fairly satisfactory results. The results obtained by the colori-

[1] Cornell University Agr. Expt. Sta. Memoir 179, June 1935.

metric method are not satisfactory, and it is believed that they are due to the failure of the analyst to prepare a good standard curve. Before the method is resubmitted for collaborative work, the instructions covering the preparation of the standard curve will be revised in order to eliminate any possibile ambiguities.

Appreciation is expressed to E. E. Mair of the H. J. Heinz Company; A. H. Robertson, State Food Laboratory, New York; A. H. Johnson, Sealtest, Inc., Baltimore, Md.; D. A. Magraw, American Dry Milk Institute, Chicago; F. B. Jones, Food and Drug Administration, New York; I. S. Shupe, Food and Drug Administration, Kansas City, Mo.; and Doris H. Tilden, Food and Drug Administration, San Francisco, for their splendid cooperation.

It is recommended that the work be continued.

REPORT ON TESTS FOR PASTEURIZATION
OF DAIRY PRODUCTS

PHOSPHATASE TEST IN EXAMINATION OF MILK AND CREAM
II. A TECHNIC FOR USE IN THE FIELD

By F. W. GILCREAS (Division of Laboratories and Research,
New York State Department of Health,
Albany, N. Y.), *Associate Referee*

Two modifications of the phosphatase test for the detection of pasteurization of milk and cream developed in the chemical laboratories of the Department of Health of the City of New York have been described by Scharer.[1,2] One of these tests is a simple technic for use in the field, which the author reports as having an accuracy comparable to that of his laboratory test or of the Kay and Graham technic.[3] In both procedures the phenol liberated as a result of hydrolysis of disodium phenyl phosphate by the enzyme is estimated colorimetrically by the addition of an alcoholic solution of 2,6 dibromoquinonechloroimide.

Because of the importance of a field test in the control of pasteurization, a collaborative study similar to that made of the modified Kay and Graham test was undertaken by the Associate Referee, *This Journal*, 21, 372–380 (1938), to determine its limitations when used by a number of different laboratory workers. Since the test had been revised subsequent to its publication, Mr. Scharer was requested to designate the technic to be followed. In order to eliminate as far as possible variations in the reagents used, he also supplied the disodium phenyl phosphate and the 2,6 dibromoquinonechloroimide in tablet form, together with color

[1] *J. Dairy Sci.*, 21, 21–34 (1938).
[2] *J. Milk Tech.*, 1, 35–38 (1938).
[3] *J. Dairy Research*, 6, 191–203 (1935).

standards corresponding to 0.2 per cent and 0.5 per cent of added raw milk, or the equivalent deficiencies in heat treatment.

PHOSPHATASE TEST FOR USE IN THE FIELD

REAGENTS

(a) *Buffer substrate.*—Dissolve 1.09 grams of disodium phenyl phosphate (washed with ether to remove free phenol and dried in a desiccator) in 900 cc. of distilled water previously saturated with $CHCl_3$. Add 50 cc. of borate buffer solution and dilute to 1 liter. Add 10 cc. of $CHCl_3$ and store in refrigerator. Or, dissolve in 50 cc. of distilled water one tablet containing disodium phenyl phosphate, $MgSO_4$, and $NaBO_2$ buffer to give 50 cc. of buffer substrate solution.

(b) *Borate buffer solution.*—Dissolve 28.427 grams of $Na_2B_4O_7 \cdot 10H_2O$ (analytical grade) in 900 cc. of warm distilled water. Stir vigorously while powder is being added to prevent lumping. Add 3.27 grams of NaOH in the form of a strong solution (2–5 N), cool, and dilute to 1 liter.

(c) *2,6 Dibromoquinonechloroimide (BQC) solution.*—Dissolve 0.04 gram of 2,6 dibromoquinonechloroimide in 10 cc. of 95% ethyl or methyl alcohol. *Do not use denatured alcohol.* Store in refrigerator. Or, dissolve in 5 cc. of 95% ethyl or methyl alcohol one tablet containing sufficient 2,6 dibromoquinonechloroimide to yield a solution of the correct concentration.

(d) *Normal butyl alcohol, neutral.*—To 100 cc. of normal butyl alcohol add 0.1–0.2 cc. of 0.1N NaOH or an amount sufficient to give a pale blue color when tested with bromothymol blue indicator. (Some normal butyl alcohol may not require neutralization.)

PURIFICATION OF BUFFER SUBSTRATE SOLUTION

Crush a buffered substrate tablet in test tube and dissolve in 5 cc. of distilled water. Add 2 drops of the BQC solution. Allow 5 minutes for color development, then extract the indophenol with 2–2.5 cc. of normal butyl alcohol. Allow to stand until alcohol layer has separated at top of tube. Remove alcohol layer with medicine dropper and discard. Dilute remainder of solution to 50 cc. This solution is then phenol-free.

PERMANENT COLOR STANDARDS

(a) *Red.*—Dissolve 59.59 grams of $CoCl_2 \cdot 6H_2O$ in and dilute to 1 liter with 1% of HCl W/V.

(b) *Blue.*—Dissolve 62.43 grams of $CuSO_4 \cdot 5H_2O$ in and dilute to 1 liter with 1 % of HCl W/V.

(c) *Yellow.*—Dissolve 45.05 grams of $FeCl_3 \cdot 6H_2O$ in and dilute to 1 liter with 1% of HCl W/V.

Prepare permanent color standards corresponding to 0.2% and 0.5% raw milk added to pasteurized milk by combining the quantities of color solutions a, b, and c, indicated in the following table and diluting to 5 cc. with distilled water in each case.

Preparation of standards

		Color Solution	
Added Raw Milk	Red	Blue	Yellow
	(a)	(b)	(c)
per cent			
0.2	0.4	1.5	0.5
0.5	0.2	2.2	0.5

PROCEDURE

Add 0.5 cc. of sample to 5 cc. of buffered substrate. Shake briefly. Incubate 10 minutes in a water-bath at 98° F. (If no water-bath is available, incubate in vest pocket for somewhat longer period.) Remove from bath, and add 6 drops of the BQC solution. Shake well immediately.

Properly pasteurized milk should give a gray or brown color reaction; properly pasteurized cream, gray or white. Raw milk or cream reacts with an intense blue color. The appearance of any blue is indicative of improper pasteurization, the intensity of color being proportional to the degree of inadequate heat treatment.

After development of color as above, add 2 cc. of normal butyl alcohol (neutral). Invert the test tube *slowly* at least 10 times and allow to stand until separation of the alcohol layer is complete. (Rapid inversion will result in formation of an emulsion. The alcohol should separate clearly and should extract the indophenol formed by the reaction.) Compare the color in the alcohol layer with the permanent standards against an opaque milk-glass plate to diffuse light through standards and sample. The appearance of any blue or blue-green in the alcohol layer is indicative of improper pasteurization. In the absence of properly pasteurized milk to be used as a control, a boiled milk may be substituted.

PRECAUTIONS

Thoroughly wash and rinse all equipment before re-use. Avoid the use of phenolic resin bottle closures anywhere in the test; the BQC reagent is sufficiently sensitive to demonstrate the leaching of phenol from the resin by water.

The following laboratories were asked to examine, beginning June 27, 1938, 6 weekly series of 12 samples each. Some laboratories were unable to examine the complete series.

Bureau of Milk Sanitation, New York State Department of Health, Albany (Mobile Laboratories, Nos. 1 and 2), W. D. Tiedeman, Chief.

Bureau of Laboratories, Connecticut State Department of Health, Hartford, F. L. Mickle, Director.

South Dakota State Chemical Laboratory, Vermillion, Guy Frary, State Chemist.

Division of Laboratories and Sanitation, Jacksonville City Department of Health, Jacksonville, Fla., H. N. Parker, Director.

Division of Foods and Drugs, Massachusetts State Department of Health, Boston, Hermann Lythgoe, Director.

Division of Chemistry, Bureau of Laboratories, Baltimore City Health Department, Baltimore, Md., Emanuel Kaplan, Chief.

Sealtest Laboratories, Baltimore, Md., Jas. J. Johnson.

Best Foods, Inc., Bayonne, N. J., H. W. Vahlteich, Chemist.

Department of Dairy Technology, Ohio State University, Columbus, L. H. Burgwald, Associate Professor.

Geneva City Laboratory, Geneva, N. Y., R. S. Breed, Director.

Tompkins County Laboratory, Memorial Hospital, Ithaca, N. Y., B. F. Hauenstein, Director.*

Chemical Laboratory, New York City Department of Health, New York, Harry Scharer, Chemist.

Division of Public Health Methods, National Institute of Health, Washington, D. C., F. J. Moss, Sanitary Engineer.

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* Dr. Hauenstein resigned as Director on July 1, but made arrangements for the examination of further series of samples.

TABLE 1.—Observed readings of all samples by comparison with color standards

SAMPLE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	a	b	c
M 30'/143°																					
701, 703, 709	bbb	aaa		aaa	sab	aaa	aab	aab	aaa		aaa	bab	aaa	aab	aaa	bbb	bbc		27	14	1
1201, 1203, 1208	cbc	aaa	sab	aaa	aaa	ooo	bab	aaa	aaa	abb	aaa	aaa	sab		aaa	bbb	aaa	sab	35	11	5
Grand total																			62	25	6
M 25'/143°																					
1103, 1108	ba	aa	aa	aa	ab	bb	aa	ab	ba	ba	aa	aa	aa	aa	aa	bb	aa	bb	25	11	0
M 20'/143°																					
702, 704, 711	bbb	ba	bb	baa	aaa	aaa	bbc	bbb	bab	cc	aaa	bba	baa	bbb	aaa	bbc	bcb	bb	18	21	4
909, 912	ac	baa	bba	aaa	c	ba	bb	bb	aa	baa	aa	bb	aa	bb	aaa	bb	aa	bbb	13	16	1
1104, 1107, 1112	abb	aa	bb	aaa	bbb	bbb	aaa	abb	bbb	baa	bab	abc	baa	bbb	aaa	bbb	aaa	bb	20	33	8
1204, 1207	cc				aa	-c	bc	bcb	aa	bc	aa	bb	ba		aa	bc	cc	bb	13	12	1
Grand total																			64	82	16
M 15'/143°																					
705, 712	cc	b	c	ba	bb	bb	cc	cc	bb	c	bb	ba	bb	cc	ba	cc	cc	c	3	13	12
1202	c		c	a	c	c	c	b	b		b	b	b		a	c	c		2	6	8
Grand total																			5	19	20
M 30'/143° +0.2% R																					
802, 805, 810	ooo	baa	occ	aaa	bac	abb	ccc	ccc	bbb	obb	bbb	aab	aaa	ccc	aba	ccc	obb	bbb	14	16	18
902, 907, 911	acc	aaa	aa	aa		bba	bbb	obb	aaa	cbc	aaa	abb	aaa	bbb	aaa	obb	aab	bb	21	19	8
1102, 1105	cb	bb				bb	aaa	bb	bo	ba	bb	aa	ab	cc	obb	bo	cb		13	17	6
Grand total																			48	52	32
M 30'/143° +0.5% R																					
903, 905, 910	ccc	bba	occ	ccc	ccc	bbb	ccc	ccc	bbb	cbc	bbb	bbc	aaa	cob	bba	ccc	bcb	ooo	5	16	27
M 30'/143° +2% R																					
706, 708, 710	ccc	aaa		ccc	ccc	ccc	ccc	ccc	ccc	cbc	ccc	ccc	ccc	ccc	cbb	ccc	ccc	ooo	0	2	40
M R																					
707	c			c	c		c	c	c		c	c	c	c	c	c	c		0	0	14
M 30'/142°																					
804, 809, 811	bcb	baa		aaa	cbs	bbb	cbc	bbb	aaa	acb	aaa	abb	aaa	bbb	aaa	bcb	aaa	bb	23	19	6
1008, 1011	ba	aa	aa	aa	aa	bb	cc		aa	bb	aa	ab	aa	bb	aa	bb	bb		20	12	2
Grand total																			43	31	8

TABLE 1.—*Observed readings of all samples by comparison with color standards*—(*Continued*)

SAMPLE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	TOTAL a	TOTAL b	TOTAL c
M 25'/142°																					
803, 812	cb	ba		aa	cb	ab	cc	bc	ab	cc	ab	ab	aa	bb	aa	cb	bb		11	13	8
1001, 1004, 1009	ccc	bbb	abb	baa	aaa	bbb	ccc		aab	bbc	abb	aab	abb	bbb	aaa	bbb	bcc	cbb	15	26	10
Grand total																			26	39	18
M 30'/142°+0.2% R																					
1005, 1007	cc	bb	bb	cc	aa	ba	cc		ab	bc	bb	ac	aa	cc	ba	bc	cc	cc	10	11	13
LC 30'/145°																					
806, 808	bb	aa	a-a	aa	ac	aa	cb	aa	aa	bb	aa	aa	aa	aa	aa	ab	aa	aa	24	6	2
LC 30'/143°																					
1205, 1209, 1211	bca	aaa	a-a	aaa	aab	bab	aaa	aaa	aaa	baa	aaa	aaa	aaa		aaa	abb	aaa	aaa	42	7	1
LC 15'/143°																					
1210, 1212	cc	aa	cb	aa	aa	bb	cb	bb	aa	bb	aa	aa	bb	aa	aa	cb	bb	bb	16	14	4
LC 25'/142°																					
1006, 1008, 1010	ccc	ccc	ccc	cbc	bba	ccc	ccc		ccc	ccc	ccc	bcc	ccc	ccc	ccc	ccc	ccc	ccc	1		44
LC 20'/142°																					
1002, 1012	cc	cc	cc	cc	cb	cc	cc	bb bb	cc	cc	cc	cc	cc	cc	cc	cc	cc	ccc	0	1	33
LC 30'/143°+0.2% R																					
901, 904	bc	aa	cc	cc	b	aa	aa	bb	aa	ac	ba	aa	aa	bb	aa	bb	ab	aa	19	9	4
1109, 1111	cb	aa	aa	aa		ab	aa	bb	bb	aa	ab	ab			aa	bb	ca	bb	16	13	2
Grand total																			35	22	6
LC 30'/143°+0.5% R																					
906, 908	cc	aa	cc			bb	ac	bc	ba	ab	bb	aa	aa	bb	aa	bb	bc	bc	11	13	8
HC 30'/145°																					
801, 807	aa	aa		aa	aa	aa	bb	aa	aa	aa	aa	aa	aa	aa	aa	aa	aa	aa	30	2	0
HC 30'/143°																					
1101, 1106, 1110	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aaa	aab	aaa	aaa	52	1	0
HC 15'/143°																					
1206	b	a	a	a	b	a	a	a	a	b	a	a	a	a	a	a	a	a	13	3	0

a = <0.2
b = ≧0.2, <0.5
c = ≧0.5

M = Milk
LC = Light Cream
HC = Heavy Cream
R = Raw

TABLE 2.—Interpretations of the examination of all samples

SAMPLE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	P	S	U
M 30'/143°																					
701, 703, 709	SSS			PPP	PPP	PPP	PPP	PPP	PSS		PPP	SPP	PPP	PPP	PPP	PPP	SSP		34	8	0
1201, 1203, 1208	UUS	PPP		PPP	PPP	UUU	PPP	PPP	PPP	PSS	PPP	PPP	PPP		PPS	PPP	SSS	PPS	35	8	5
Grand total																			69	16	5
M 25'/143°																					
1103, 1108	UP	PP		PP	PS	UU	PP	PS	US	SS	PP	PP	PP	PP	SP	PP	SS		20	8	4
M 20'/143°																					
702, 704, 711	SSS	UP		UPP	SSP	PPP	PPS	SSS	SPS	UU	PPP	SSP	PPP	SSS	PPP	PPS	SUS	SS	21	19	2
909, 912	PU	SPP			−U	SP	PP	SS	PS	SSS	PP	SS	PP	SS	SS	PP	SS	SSS	12	14	5
1104, 1107, 1112	PPS	SPP		PPP	SUU	UUS	PPP	SSS	UUU	SSS	SPS	PSU	PPP	SSS	PPP	PPP	UUU	SSS	21	19	11
1204, 1207	UU	PS		PP	PP	−U	PS	SS	SS	SU	PP	SS	PP	SSS	SS	PS	UU	SS	11	14	6
Grand total																			65	66	24
M 15'/143°																					
705, 712	SS	S		UP	UU	SS	SS	SS	UU		SS	SP	SS	UU	US	SS	UU		2	16	10
1202	U			P		U	S	S	S	U	U	U	P		S	S	U	U	2	6	7
Grand total																			4	22	17
M 30'/143°+0 2%R																					
802, 805, 810	SSS	UPS		PSP	USU	PSS	UUU	SSS	UUU	USS	USS	PPS	PPP	UUU	SSP	SSS	USS	SSS	10	23	15
902, 907, 911	PUU	PPP			SP	UUP	PPP	SSS	SPS	USU	PPP	PSS	PPP	SSS	SPS	SPP	SSU		19	19	7
1102, 1105	UU	UU		PP		UU	PP	SS	UU	SS	US	PP	PP	SS	PS	PS	UU		11	12	11
Grand total																			40	54	33
M 30'/143°+0.5% R																					
903, 905, 910	UUU	SSP		UUU	UUU	UUU	UUU	UUU	UUU	USU	UUU	SSU	PPP	UUU	UUS	SSS	UUU	USU	4	11	30
M 30'/143°+2% R																					
706, 708, 710	UUU			U	UUU	UUU	UUU	UUU	UUU		UUU	UUU	UUU	UUU	UUU	UUU	UUU		0	0	42
M R																					
707	U			U	U	U	U	U	U	U	U	U	U	U	U	U	U		0	0	14
M 30'/142°																					
804, 809, 811	SSS	SPP		PSS	USS	SSS	SPU	SSS	SSS	SUS	PPP	PSS	PPP	SSS	SPS	PSP	SSS		14	31	3
1008, 1011	SP	PP	PP	PS	PP	PP	UU		SP	SS	PP	PS	PP	PP	SS	PP	UU	SS	20	10	4
Grand total																			34	41	7

TABLE 2.—*Interpretations of the examination of all samples*—(*Continued*)

SAMPLE	LABORATORY NUMBER																		TOTAL		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	P	S	U
M 25'/142°																					
803, 812	SS	UP		PP	UU	PS	UU	SS	PU	UU	PS	PS	PP	UU	SP	SP	SU		11	10	11
1001, 1004, 1009	USS	SSS	PSS	UPS	PPP	USU	UUU		SSU	SSU	PSS	PPS	PSS	SSS	SSS	PPP	UUU	SSS	12	27	12
Grand total																			23	37	23
M 30'/142°+0.2% R																					
1003, 1007	SP	SU	SS	UP	PP	UP	UU	SS	PU	UU	UU	PU	PP	UU	US	PS	UU	SU	9	9	16
LC 30'/145°																					
906, 908	SS	PP		UP	SU	PP	SP	SP	SU	SS	PP	PP	PP	PP	US	PS	PP	SU	24	7	1
LC 30'/143°																					
1205, 1209, 1211	USP	PPP		PPP	PPS	UPS	PPP	PPP	PPP	SPP	PPP	PPP	PPP		PPP	PPP	SSS	PPP	39	7	2
LC 15'/143°																					
1210, 1212	SS	PP		PP	PP	SS	PP	SS	SS	SS	PP	PP	PP		PP	SP	UU	SS	17	13	2
LC 25'/142°																					
1006, 1008, 1010	USU	UUU	UUU	UUU	SSP	UUU	UUU		UUU	UUU	UUU	SUU	UUU	UUU	UUU	UUU	UUU	UUU	1	4	46
LC 20'/142°																					
1002, 1012	UU	UU	UU	UU	UU	UU	UU		UU	UU	UUU	UU	UU	UU	UU	UU	UU	UU	0	0	34
LC 30'/143°+0.2% R																					
901, 904	UU	PP		PP		PP	PP	SS	SS	PU	PS	PP	PP	PP	PP	PP	SU	PP	21	5	4
1109, 1111	UU	PP			S—	PS	PP	SS	UU	PP	PS	PS		SS	SS	PP	US	SS	13	13	5
Grand total																			34	18	9
LC 30'/143°+0.5% R																					
906, 908	UU	PP		PP	PS	UU	PU	SU	US	PS	UU	PP	PP	UU	PP	PP	UU	SU	12	4	14
HC 30'/145°																					
801, 807	PP	PP		PP		PP	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP	PP		31	1	0
HC 30'/143°																					
1101, 1108, 1110	PPP	PPP		PPP	PPP	PPP	PPP	PPP	PPP	SSP	PPP	PPP	PP—	PPP	PPP	PPP	SPP	PPP	47	3	0
HC 15'/143°																					
1206	U	P		P	S	P	P	P	P	S	P	PPP	P		P	P	P	P	13	2	1

M = Milk
LC = Light Cream
HC = Heavy Cream
R = Raw

P = Pasteurised
S = Slightly Underpasteurised
U = Underpasteurised
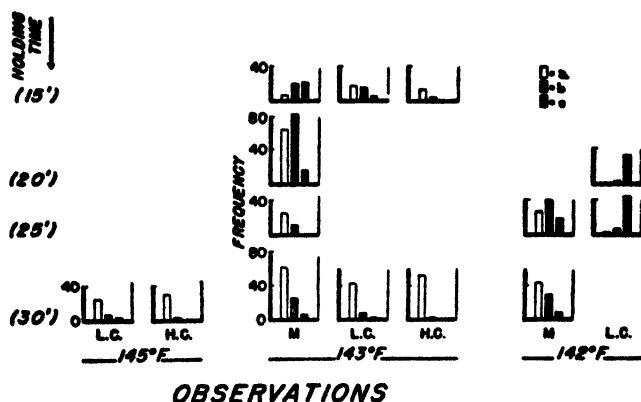
FIG. 1.—FREQUENCY DISTRIBUTION OF OBSERVED READINGS BY COMPARISON WITH COLOR STANDARDS. SAMPLES HEATED TO DESIGNATED TEMPERATURE AND TIME.

a = <0.2; b = ≧0.2 and <0.5; c = ≧0.5
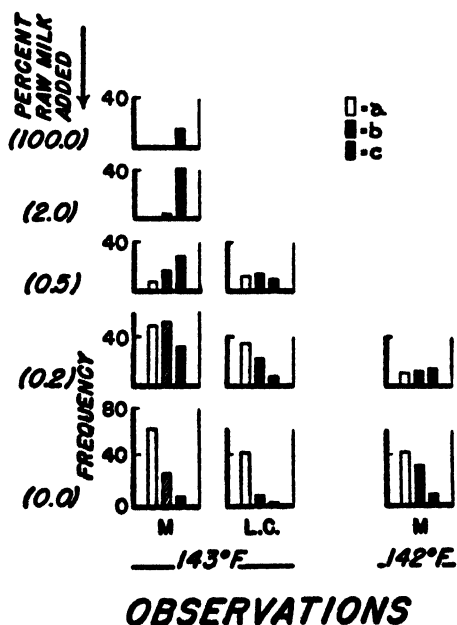
FIG. 2.—FREQUENCY DISTRIBUTION OF OBSERVED READINGS BY COMPARISON WITH COLOR STANDARDS. PASTEURIZED MILK AND CREAM WITH ADDED RAW MILK.

a = <0.2; b = ≧0.2 and <0.5; c = ≧0.5

Food Research Division, United States Department of Agriculture, Washington, D. C., Walter S. Hale, Associate Chemist.

City Health Department, Chicago, Ill., Herman N. Bundesen, President of the Board.

New Jersey Agricultural Experiment Station, New Brunswick, O. F. Garrett, Assistant in Dairy Manufacture.

The samples represented milk taken at various holding periods of commercial pasteurization at 143° F.; milk pasteurized at 142° F. in the Referee's laboratory; and also pasteurized milk or cream to which had been added definite quantities of raw milk. The samples were packed in sufficient dry ice to provide 6–10 hours of refrigeration without freezing and were shipped for delivery within 24 hours by parcel post, special delivery, or by air express to the more distant points. Each sample was identified by a serial number only.

The cooperating laboratories were requested to report their readings as equal to, less than, or greater than the standards and also to state their interpretation of the treatment of each sample as pasteurized, slightly underpasteurized, underpasteurized, or grossly underpasteurized. The results of the examinations by all laboratories reporting the readings in comparison with the color standards are given in Table 1 and the interpretation of results in Table 2. Figures 1 and 2 show graphically the frequency distribution of the observed results obtained by comparison with the color standards, for milk and cream heated to the given temperature and for the given period, and for pasteurized milk containing the stated percentage of added raw milk. Figures 3 and 4 show graphically the frequency distribution of the designated interpretation of the results of the examinations of the same samples.

Inspection of these results indicates that unheated milk and a pasteurized product containing 2 per cent of added raw milk were accurately determined in every case. In the examination of the 90 samples of properly pasteurized milk, 16 were reported as underpasteurized and 5 as grossly underpasteurized. Similarly, of the 93 samples of pasteurized milk examined by the extraction technic and compared with the color standards, 31 were reported as having a color >0.2 standard and thus as improperly treated. In the examination of 44 samples of milk heated for 15 minutes at 143° F., 5 were reported as properly pasteurized. Of 180 samples of pasteurized milk containing added raw milk in concentrations of less than 2 per cent, 53 were designated as pasteurized. Lowering the temperature of the heating of 165 samples was detected with an accuracy of 58 per cent.

From the results reported by the various cooperating laboratories, it is apparent that in the examination of cream the high percentage of butter fat interferes with the color reactions and thus renders the application of this test to such samples difficult. This probably accounts for the tendency to report a relatively large proportion of samples of underpasteurized cream as pasteurized.
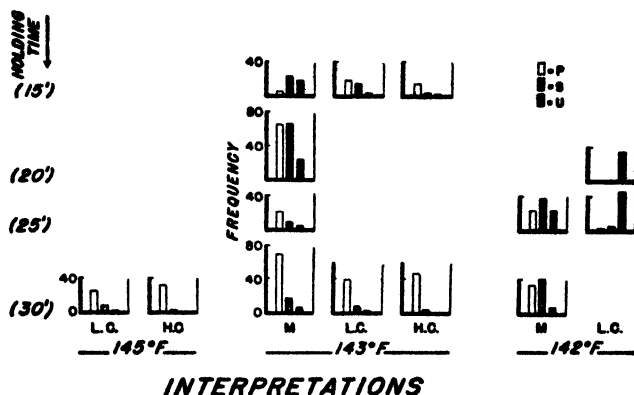
FIG. 3.—FREQUENCY DISTRIBUTION OF INTERPRETATIONS OF RESULTS. MILK AND CREAM HEATED TO DESIGNATED TEMPERATURE AND TIME.

P = pasteurized; S = slightly underpasteurized;

U = underpasteurized or grossly underpasteurized.
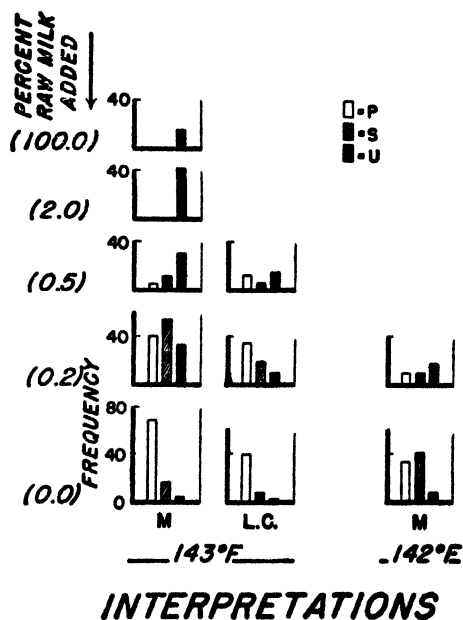
FIG. 4.—FREQUENCY DISTRIBUTION OF INTERPRETATIONS OF RESULTS. PASTEURIZED MILK AND CREAM WITH ADDED RAW MILK.

P = pasteurized; S = slightly underpasteurized;

U = underpasteurized or grossly underpasteurized

SUMMARY

The results of this collaborative study by 18 laboratories in examinations with identical samples, technic, and reagents, indicate that the phosphatase field test of Scharer should be subjected to further study for possible modifications toward the attainment of maximum accuracy.

Based on the data presented, the following recommendations[1] are made:

(1) That action be deferred on the adoption of the phosphatase field test as developed by Scharer.

(2). That further studies be made in order that a suitable, precise, and sensitive technic for field use in the control of pasteurization may be developed.

The assistance of Dr. William R. Thompson in the development of the graphic representation of the results was of great value in the preparation of this report and is appreciatively acknowledged.

No report on citric acid in milk was given by the Associate Referee. He stated that this project would be postponed until a method for the determination of citric acid in fruits and fruit products had been formulated.

REPORT ON DIFFERENCE BETWEEN DAIRY PRODUCTS MADE FROM COW'S MILK AND THOSE MADE FROM THE MILK OF OTHER ANIMALS

By IRA D. GARARD (New Jersey College for Women, Rutgers University), *Associate Referee*

Several dairy products made from the milk of sheep or goats are sold widely in the United States. It seems desirable, therefore, from the viewpoint of legal control and trade practice, that there be some recognized method for distinguishing these products from similar ones made from cow's milk.

There are few published analytical data on dairy products other than those made from cow's milk. However, Currie[2] in a study of Roquefort cheese reported Polenske values of 5.55, 6.25, 5.68, and 5.6, respectively, for the fat from four brands of this cheese. Except these results and a few other Polenske values of the same order, but widely scattered throughout the literature, almost no other data were available until Garard, Minsky, Baker, and Pascale[3] published the results of an investigation of the ap-

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 60 (1939).
[2] *J. Agr. Research*, 2, 1-14, 429-84 (1914).
[3] *Ind. Eng. Chem.*, 29, 1167-71 (1937).

TABLE 1.—*Collaborative results*

Collaborator	A				B				D				E			
METHOD	Reichert-Meissl I	II	Polenske I	II	Reichert-Meissl I	II	Polenske I	II	Reichert-Meissl I	II	Polenske I	II	Reichert-Meissl I	II	Polenske I	II
Burton Jordan	25.6	25.7	1.6	2.0	22.8	23.0	1.8	2.1	29.1	29.1	3.4	(2.65)*	30.8	30.3	4.6	3.2
State Chemical Lab.	25.7	26.1	1.5	1.9	23.1	22.9	2.0	1.8	29.2	29.6	3.5	4.2	30.8	30.7	4.4	5.1
Vermillion, S. D.	25.9	26.2	1.8	1.9	23.2	23.1	1.9	2.3	29.5	29.4	3.3	4.1	30.8	30.7	4.6	(1.10)*
					23.1		1.6			29.3		4.6		30.5		4.6	
					23.4		1.8										
					22.9											
Ira D. Garard	26.2	25.5	1.9	2.4	23.1	23.0	2.1	2.7	27.2	27.5	3.0	4.4	29.9	29.6	4.3	5.2
	25.0	24.7	1.8	2.5	22.8	23.2	1.9	2.8	26.7	26.5	3.4	4.5	29.7	30.4	(6.4)*	4.5
K. R. Majors	27.1	26.6	2.1	2.1	22.8	23.4	1.8	2.7	29.0	28.8	3.7	4.6	30.3	29.6	5.4	5.0
U. S. Regional Soybean	27.0	26.3	2.0	2.4	22.6	23.2	1.8	3.0	29.0	29.3	3.2	3.9	29.3	30.2	4.7	5.0
Ind. Products Lab.	27.0		1.9							29.4		3.6	29.8		4.0	
Urbana, Ill.													29.6		4.6	
R. S. McKinney	25.3	25.6	1.4	1.7	22.7	22.0	1.9	1.8	28.1	27.4	(2.45)*	3.7	29.1	28.9	3.6	4.5
Bureau of Chemistry and Soils	25.1	25.7	1.4	1.8	22.5	22.3	1.9	1.9	28.3	27.4	3.1	3.7	29.3	29.1	3.6	4.5
Washington, D. C.		25.8		1.9		22.3		2.2	28.5		3.8		29.8		3.7	
						22.9										
J. L. Perlman	25.9	26.7	2.1	2.2	21.9	22.9	2.4	2.8	29.3	29.6	3.6	4.8	30.1	30.2	4.0	5.3
State Food Lab.	26.5	26.2	2.1	2.4	22.5	22.1	2.2	2.5	28.9	29.3	3.4	4.6	30.0	29.8	4.1	5.2
Albany, N. Y.																
M. L. Offutt	25.9	25.6	1.6	1.8	22.6	23.2	2.0	2.0	28.2	28.4	4.0	4.8	29.9	30.0	4.1	4.7
U. S. Food and Drug Adm. Lab.																
New York																
F. H. Lehlberg	26.2	26.3	2.5	2.6	23.8	23.4	2.4	2.6	30.1	30.2	5.1	5.1	30.5	30.2	4.2	5.1
Grain Research Lab.																
Winnipeg, Man.																
Mean	26.0	25.9	1.8	2.1	22.9	22.8	2.0	2.4	28.7	28.7	3.6	4.3	30.0	30.0	4.4	4.8

* Not included in averages.

plicability of the Polenske value to the distinction of Roquefort cheese from similar varieties made from cow's milk.

As Currie's work and the results on the percentage distribution of acids in the fats by Dhingra[1] and by Crowther and Hynd[2] indicate the usefulness of the Polenske value, and because of the amount of work involved, no other constant was investigated by these workers. The literature, however, was searched comprehensively and 28 loaves of Roquefort cheese of different years and brands were analyzed, together with over a hundred samples of other varieties of American and foreign cheese, in order to establish the variations to be expected from samples of known origin. This work appears to show that the Polenske value distinguished between Roquefort cheese that gave values from 3.6 to 5.95 and blue cheese from cow's milk that had not been found to give values in excess of 3.

All the results reported in this investigation were obtained by the four authors and most of them by two of the four. Consequently, the method of procedure in the determinations was fairly uniform. As the results found in the literature were obtained by Polenske methods of unknown detail, it was considered that collaboration would be desirable in order to ascertain the cause of the variability of results obtained by other analysts under the conditions of the official method.

Samples of fat were extracted from the cheese by the Associate Referee and sent to chemists who had consented to do collaborative work on these determinations. The work was a joint project with R. S. McKinney, who was making a study of the effect of pumice size on the results by the Polenske method. Although five samples were sent to collaborators, one was not a pure cheese fat and so the results from only four of them are included in this report. Each sample was analyzed by the official Polenske method (Method I) and also by the same method with 0.1 gram of powdered pumice (Method II) instead of the "few pieces" specified in the official method. Sample A was the fat from a loaf of Royal Beech, Danish Blue Cheese, and B was from Flora Danica, Danish Blue Cheese. Sample D was from Societé Bee brand and E from Puma brand, Roquefort cheese. All samples were extracted by the acid extraction method, *Methods of Analysis*, A.O.A.C., 1935, 292, 99(b), and washed free from acid. The results obtained by the collaborators are given in Table 1.

Table 1 contains all the results submitted, but the four values inclosed in parentheses are not included in the averages. Three of these are results obtained by Method II, with powdered pumice, and in each of the four cases the deviation of the discarded result was more than four times the mean deviation of the other results on the same sample. In one instance (Lehlberg) the results are the average of three or more determinations.

[1] *Biochem. J.*, **27**, 851–59 (1933).
[2] *Ibid.*, **11**, 139–63 (1917).

DISCUSSION OF RESULTS

Sixty-one Polenske values on two samples of fat from blue cheese vary from 1.4 to 2.8 and give a mean value of 2.0; 59 Polenske values on two samples of fat from Roquefort cheese vary from 3.0 to 5.4 and give a mean value of 4.1.

The probable error of a single determination was calculated for each cheese separately, but the results of both methods of analysis are included. For example, the mean value for Sample A was calculated from all the Polenske values in Columns I and II in Table 1. These results are shown in Table 2.

TABLE 2.—*Probable error*

CHEESE FAT	NUMBER OF DETERMINATIONS	MAXIMUM AND MINIMUM	MEAN POLENSKE VALUE	PROBABLE ERROR
Royal Beech, Blue	30	2.6–1.4	2.0	0.22
Flora Danica, Blue	31	3.0–1.6	2.2	0.26
Societé Bee, Roquefort	29	5.1–3.0	4.0	0.42
Puma, Roquefort	30	5.4–3.2	4.5	0.38

In view of the bumping that occurred in several determinations with Method II, the probable errors are not large.

The Reichert-Meissl values have never been suggested as being significant in this problem, probably because values for the fat of cow's milk have been reported at least as high as 33. However, the literature has not been thoroughly searched for these values for cheese fat. The values obtained in this collaborative work are interesting and may be significant. Sixty-seven values on two samples of fat from the cow's milk cheese vary from 21.9 to 27.1, with an average value of 24.3; 67 values from two samples of fat from Roquefort cheese vary from 26.5 to 30.7, with an average value of 29.3. From these results it would seem that the usefulness of the Reichert-Meissl value deserves further investigation.

The Polenske values are in agreement with similar values found in the literature, and it appears to be well established that if two or more determinations give a mean Polenske value less than 3 for the fat of a cheese, the cheese has not been made from ewe's or goat's milk or a mixture of these two milks.

The Associate Referee wishes to express his appreciation of the thorough work of his collaborators and their excellent cooperation.

RECOMMENDATIONS[1]

It is recommended—

(1) That a study be made of the application of the Polenske method to the identification of cheese from goat's milk and to products from milk mixtures.

[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 60 (1939).

(2) That the other fat constants of milk and cheese fat, particularly the Reichert-Meissl and Kirschner values, be studied with reference to their application to these problems.

No report on naval stores was given by the Referee.

No report on rosin was given by the Associate Referee.

REPORT ON TURPENTINE

By V. E. GROTLISCH (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The report of the Associate Referee on Turpentine last year pointed out that there was possibility of the need of new official methods for detecting and determining adulteration of turpentine with certain new hydrogenated petroleum oil thinners, which behave very much like some of the coal tar oils. No evidence or indication, however, of adulteration of turpentine with such products has come to the attention of the Associate Referee during the year. On the other hand, there have been several cases of adulteration of gum spirits of turpentine with steam distilled wood turpentine and with sulfate wood turpentine.

Sulfate wood turpentine is recovered as a by-product in the manufacture of paper pulp from resinous wood by the sulfate process, in which the wood is subjected to the action of a solution containing sodium hydroxide, sodium sulfide, and sodium sulfate. The vapors from the digesters are condensed and yield a very foul-smelling oil, crude sulfate wood turpentine. The odor is due primarily to sulfur compounds in the nature of mercaptans. The crude is refined by a series of fractional distillations, the sulfur compounds being low boiling and concentrating in the "heads" of the distillation. However, it is practically impossible to remove all the sulfur. Some manufacturers of sulfate wood turpentine reflux the turpentine with sodium or calcium hypochlorite (chloride of lime), which removes most of the sulfur, so that ultimately the refined sulfate wood turpentine contains less than 0.03 per cent sulfur, but the hypochlorite treatment results in putting small quantities of combined chlorine in the turpentine instead of the sulfur. Aside from the distinctive odor of the sulfate wood turpentine, it is possible to prove its presence, therefore, by the detection or determination of sulfur and/or chlorine.

In a paper published several years ago, W. C. Smith[1] of the Bureau

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[1] *Ind. Eng. Chem. Anal. Ed.*, 3, 354 (1931).

of Chemistry and Soils, reported that the small quantities of these identifying elements present, ordinarily less than 0.03 per cent sulfur, and less than 0.3 per cent chlorine (the sulfur content ordinarily drops to around 0.01 per cent when the hypochlorite treatment is used) cannot be isolated or recovered in the ordinary combustion or oxidation methods. However, the sulfur and chlorine can be determined quantitatively by burning the turpentine in a carbureting device known as the Kennedy sulfur lamp,[1] in which a measured quantity (5 cc.) of the turpentine is slowly fed down into the lamp, where it is vaporized by heat from a resistance coil embedded in the lamp, and mixed with air that comes from an air line in the laboratory. The turpentine so vaporized is burned at the exit orifice or tip, and the products of combustion are absorbed in a volumetric solution of standardized sodium carbonate by placing a chimney over the flame and connecting the absorption apparatus to the laboratory vacuum line.

The sulfur burns to SO_2, and, strange to say, the chlorine in the turpentine is converted quantitatively in the combustion to hydrochloric acid.

Titration of the excess of sodium carbonate indicates the sulfur, or, if chlorine be present, the total sulfur equivalent. The presence and quantity of chlorine is determined, after titration of the excess alkali with standardized (usually less than 0.1 N) nitric acid, by washing the solution from the absorption chamber, warming, acidulating with several drops of nitric acid, and adding a solution of silver nitrate. The sodium chloride is precipitated as silver chloride which, on standing, settles, and can be quantitatively filtered and weighed. From the weight of the silver chloride, the weight and per cent of chlorine can be computed. From this, by proper allowance for the difference between the weight of chlorine and sulfur, the quantity of sulfur can be obtained by difference from the results of the first titration. The sulfur content can be checked by running another combustion and, after back titration and oxidation of the solution with bromine water, the sulfur is precipitated and weighed as barium sulfate.

Chadwick and Palkin[2] have shown that aside from its distinctive odor, steam distilled wood turpentine differs from gum spirits by the normal presence in the former of small quantities of benzaldehyde and fenchyl alcohol, neither of which has been found in gum spirits. The benzaldehyde can be extracted by repeated extractions of a sizable quantity of the turpentine with sodium bisulfite solution. After sodium carbonate has been added to liberate the aldehyde, the extracts are steam distilled, when if steam distilled wood turpentine is present in appreciable quantity in the sample, the odor of benzaldehyde should be noticeable. The

[1] *Ind. Eng. Chem.*, **20**, 201 (1928).
[2] Proc. A.S.T.M. 37, Part II, p. 574 (1937).

benzaldehyde may be extracted from the distillate with petroleum ether, and finally the benzaldehyde is identified by converting it into a crystalline addition product using 2,4 dinitrophenylhydrazine. The purified benzaldehyde, 2,4 dinitrophenylhydrazine melts at a temperature between 237° and 242° C.

Collaborative work on standard methods of detecting such sophistication of gum spirits of turpentine and determining the presence of the identifying constituents is planned.

A study is now being made, through the Sub-committee on Turpentine of the American Society for Testing Materials, of the possibility of developing a new partial-immersion sensitive thermometer for the turpentine distillation test, to replace the fully-immersed Anschutz type of thermometer now regularly used. Specifications have been developed, and several of the manufacturers have made up trial thermometers. These are now ready for testing to compare the distillation results obtained therewith with those obtained by the present standard thermometer. The initial results indicate that a sensitive partial-immersion thermometer can be manufactured at suitable cost to replace the fully immersed thermometer, which is rather difficult to read, especially at the start of a distillation test, when the turpentine contains a small quantity of dissolved water. As soon as this work is completed in the A.S.T.M., it will be brought to the attention of the A.O.A.C.

No recommendations are being made at this time, other than that the work on turpentine be continued.

REPORT ON PAINTS, VARNISHES, AND CONSTITUENT MATERIALS

By C. S. LADD (Food Commissioner and Chemist, Bismarck, N. D.), *Referee*

It is recommended[1]—

(1) That the standard methods of the American Society for Testing Materials for testing skinning and alkali resistance of varnishes (D154-38) be adopted as tentative.

(2) That study of the methods of testing abrasion resistance and hardness of varnish films be continued.

(3) That study of a method for soap resistance of varnish be made and that a study be made with a view to revising the present method of testing elasticity or toughness of varnish films in order to make execution of the method less tedious.

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 54 (1939).

(4) That study of the accelerated weathering test of paints be continued.

No report on accelerating testing of paints was given by the associate referee.

REPORT ON VARNISHES

By F. ROBERTS (Paint and Varnish Laboratory,
Bismarck, N. D.), *Associate Referee*

No significant developments in methods for varnish analysis and tests have been reported during the past year, but work along these lines has been planned for the coming year.

In the present method for determining elasticity or toughness of varnish films considerable time is involved due to the trial and error nature of the procedure in ascertaining the amount of run kauri solution (within 10 per cent), to add to the product to cause a break in the film when bent over a ⅛ inch mandrel. It is planned to make investigations with a cone-shaped mandrel with which it may be possible to estimate the elasticity by determining the greatest diameter on the cone where cracking occurs under certain reductions. Considerable time may possibly be saved if a satisfactory procedure can be developed through the use of a conical mandrel in making this test.

It has been suggested that a soap resistance test would be of value in determining durability of interior finishes, especially floor varnishes. Work along this line will be done in an effort to develop a suitable practical method for making such a test.

Work on abrasion resistance will be continued with the end in view of developing a satisfactory method that may be standardized.

The following methods are presented for adoption as tentative:[1]

SKINNING TEST OF VARNISH IN CLOSED CONTAINERS[2]

Measure a 6 ounce sample of the varnish, by means of a graduate, into an 8 ounce wide-mouthed glass jar 4½ inches in height and 2 inches in diameter. Screw the cover on tightly and invert the jar momentarily. Place the jar in an upright position in the dark (under a box or in a drawer is satisfactory) and examine the varnish for skinning after desired periods of time, such as each 24 hours for the first week and each week thereafter for a total period of four weeks.

ALKALI RESISTANCE TEST[2]

Thoroughly clean a 1×6 inch test tube in benzol. Dip the tube into the sample varnish, immediately invert the mouth of the tube, and allow the varnish to dry for 48 hours in an atmosphere free from dust, drafts, products of combustion, or laboratory fumes. Maintain a room temperature of 19°–25° during the drying

period. Suspend the varnish-coated tube in 3% NaOH solution maintained at 21°± 1.1°. Remove the tube after desired periods of time, such as 8, 16, 24, and 24 hours, rinse under a gentle stream of water, allow to air dry for 30 minutes, and examine the varnish, for whitening, blistering, or removal.

No report on leathers and tanning materials was given by the Associate Referee.

REPORT ON RADIOACTIVITY

SIMPLE AND INEXPENSIVE CHARGER FOR ELECTROSCOPES

By Anna E. Mix (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Solid or liquid substances are tested for radioactivity with alpha, beta, or gamma ray electroscopes, and it is necessary to subject these instruments to an electric charge in order to place the leaf or fiber at a definite point on the scale that is set in the microscope of the instrument. The charging is usually done by rubbing a glass rod with a piece of silk cloth or by rubbing a hard rubber rod with rabbit fur.

A refined friction device of English manufacture used in connection with the work in this laboratory became inoperative, and the inability of the manufacturers to replace this charger prompted the acquisition of a substitute for this purpose.

The use of a thermionic device suggested by Alfred Christie of the Bureau of Agricultural Economics resulted in a small simple charger that may be built for less than $5.00 by anyone having some knowledge of thermionic tubes and their operation.

A half-wave rectifier tube, 25Z5, was selected, because it doubles the input voltage of the alternating current power line and gives a high voltage without the use of transformers. A line cord resistor of 280 ohms was used to reduce the voltage and heat of the tube filament. The two condensers used in the circuit are of 8 mfd. capacity and they should withstand a surge of 250 volts without a breakdown.

A six-prong socket, single-pole single-throw switch, a double-pole single-throw switch, and a pair of test leads with the necessary base and housing complete the parts necessary for the construction of the charger.

A bleeder resistance of approximately 60,000 ohms was used across the output.

This device may also be used for removing the static charge from finely powdered drugs and other powdered substances. The ampoule or capillary containing the sample is touched first with one point of the charger while the free lead is being grounded and then with the other. The container is supported by glass rods or on a beaker while being treated.

Operation

The charger is plugged into the alternating current power line, the single-pole power switch is turned on, and the double-pole charge switch is turned off. The tube is allowed to warm up for approximately 3 minutes, which gives the condensers time to become charged. The black or
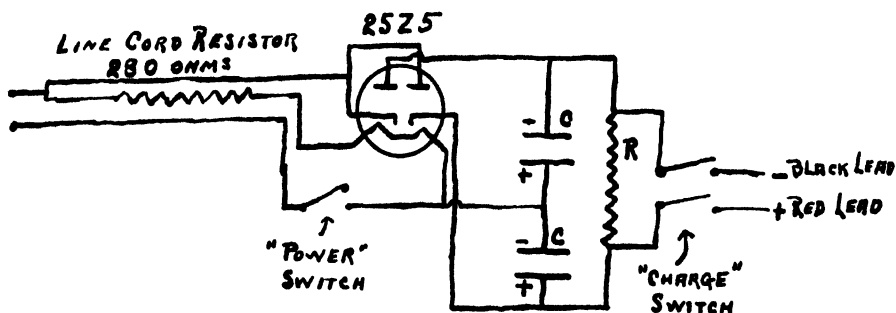
FIG. 1.—SHOWING CHARGER CIRCUIT.
R—60,000 ohm resistor.
C—8 mfd. electrolytic condenser.

negative lead is then attached to the frame of the electroscope, the charge switch is turned on, and the red or positive lead is repeatedly applied to the positive contact of the electroscope until it is fully charged. When the charger is not in constant use the charge switch is turned off and the power allowed to remain in the charger. When needed again it is then only necessary to turn the charge switch on and after completing the work with the electroscope, to turn the charger power switch off.

The Associate Referee wishes to thank Mr. Christie for his timely suggestions and for improving and reducing the cost of this instrument.

REPORT ON QUANTUM COUNTER

By ANNA E. MIX (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Within the past few years much importance has been attached to the determination of radioactivity in foods, drugs, and mechanical devices. It was necessary, therefore, to acquire a sensitive device for its detection, as even a small amount of ingested radium causes undesirable and in some cases fatal results, while on the other hand mechanical devices, claimed to be radioactive, but containing none or such minute quantities of radium that they are of no value, are classed as cheats.

The alpha, beta, and gamma ray methods described in *Methods of Analysis, A.O.A.C.*, are satisfactory and give reliable results, but they are time-consuming.

Some time ago a quantum counter was developed by R. D. Evans of the Massachusetts Institute of Technology, and one of these instruments was purchased by the Food and Drug Administration. This instrument is more rapid and is claimed to be more sensitive than the gamma ray scope. Literature states that it is 10 to 100 times more sensitive to feeble gamma rays than is the best electroscope.

Briefly, the principle of operation is as follows: A glass cylinder containing gas at a low pressure, or evacuated, is grounded. A wire runs through this cylinder and is connected to the remainder of the circuit. The wire in the cylinder is charged positively. The cylinder therefore assumes a negative charge and the difference of potential between the wire and the cylinder is kept at about 1200 volts. The cylinder screens out alpha and beta particles. However, the gamma rays ionize the surrounding air and produce a discharge from the cylinder to the wire and thence to the recording device. Every gamma ray quantum producing one or more secondary electrons inside the cathode cylinder causes the instrument to discharge once.

The circuit in the quantum counter used in this laboratory is such as to permit three types of recording, one auditory and two visual. Of the two visual recording devices, one is an electric eye, the other a rate meter (volt meter type).

Neon glow lamps are used in the circuit for voltage stabilization. A detailed report of the circuit may be found in the paper: "Quantum Counter Amplifiers for Gamma Ray Detection and Applications to Studies in Radium Poisoning," by Robley D. Evans, *Proc. Amer. Phil. Soc.*, **78**, No. 1 (Oct. 1937).

While the Associate Referee has made over 1000 readings, no report as to method of calculation of sensitivity of this scope will be given this year for it is considered that additional work is needed to obtain more reliable results. Therefore it is recommended that work on the quantum counter be continued.

No report on the gamma ray scope was given by the Associate Referee.

REPORT ON COSMETICS

By ELMER W. CAMPBELL (Department of Health and Welfare, Augusta, Maine), *Referee*

Owing to the fact that the collaborative study of cosmetics is a new project, it is rather difficult to decide where to start in this broad field to develop a worthwhile program.

Preliminary studies having shown or indicated that many lipsticks and rouges contain barium or arsenic, or possibly both, and that the probable

source of these ingredients is the dyes or coloring substances used in such preparations, the Referee suggested a study of coloring substances used in such cosmetics. Preliminary studies of a number of samples of dyes secured from various manufacturers were made.

Soluble barium salts in cosmetic colorings

LABORA-TORY	ACID-SOLUBLE Ba			WATER-SOLUBLE Ba			TOTAL SOLUBLE Ba		
	1	2	3	1	2	3	1	2	3
Sample	*per cent*			*per cent*			*per cent*		
1	0.2085	—	—	0.5065	—	—	0.715	—	4.57
2	0.0529	0.0145	—	0	—	—	0.0559	0.0145	2.65
3	0.2445	0.2646	—	0.3722	0.2881	—	0.6167	0.5527	4.30
4	0.1149	0.1146	—	0.2007	0.0017?	—	0.3157	0.1263	3.13
5	0.0557	0.1705	0.60	0.1515	0.1450	0.42	0.2072	0.3155	1.02
6	1.1656	0.6250	1.51	1.2922	0.9938	1.71	2.6240	1.6188	3.22
7	1.103	0.3163	1.42	0.8745	0.2375	1.44	1.9775	0.5538	2.86
8	2.585	0.1375	1.61	0	—	0.11	2 585	0.1375	1.72
9	0.4480	0.2675	1.06	0.1943	0.0823	0.19	0.6423	0.3498	1.25
10	7.88	3.9402	7.59	1.075	0.1805	1.32	8.955	4.1207	8.91

The method of analysis that was suggested follows. It may have been modified by the different collaborators.

BARIUM IN LIPSTICKS, CREAM ROUGES, AND LAKE DYES
Water-Soluble Barium

I. Weigh a sample in tared Pt dish and ash cautiously over a Bunsen burner.

(a) This precipitate, in case Ca was present in the original sample, is contaminated with $CaSO_4$, giving a high result in both water-soluble and HCl-soluble procedures. However, the percentage of these cases does not appear to warrant a separation by the chromate method. Possibly a spectrographic quantitation would solve this problem.

II. To the ash add 10 cc. of 3% H_2O_2, mix well, and evaporate at low temperature over a water bath to dryness. (b) To the ash add 25 cc. of water, heat to boiling, filter, wash well with hot water, and dilute the filtrate to about 200 cc. Use caution in the choice of filters. After filtering allow the filtrate to stand about 4 hours and then refilter through a double filter, as the $BaSO_4$ base used for the dyes is extremely fine and if carried through the filter would give high results.

(a) On ashing in a muffle furnace there was thought to be considerable reduction of $BaSO_4$ to BaS, thereby giving a higher percentage of water-soluble barium than was present before ashing.

(b) Even using a Bunsen burner and ashing cautiously there seemed to be some reduction. The H_2O_2 treatment is for the purpose of oxidizing the BaS back to the $BaSO_4$.

III. Add 2 cc. of dilute HCl and heat to boiling; add 2 cc. of dilute H_2SO_4 and let stand several hours, preferably overnight. If a precipitate settles out, collect on an ashless filter, place the filter in a tared ignition crucible, and ignite carefully to a white ash. Cool, and weigh as $BaSO_4$. From the weight calculate as Water-Soluble Barium (%). Check to see if free from calcium.

Dilute HCl-Soluble Barium

Ash the filter and residue from I over a Bunsen burner in the original Pt dish. Treat the ash with 50 cc. of water and 2 cc. of dilute HCl, heat to boiling, filter,

and wash the filter with hot water, using the same precautions as in II. Dilute the filtrate to 200 cc., add 2 cc. of dilute H_2SO_4 and allow the solution to stand several hours, preferably overnight.

If a precipitate settles out, collect it on an ashless filter and wash well. Place the filter in a tared crucible and ignite carefully to a white ash. Cool, and weigh as $BaSO_4$. (a) From this weight calculate as percentage of dilute HCl-Soluble Barium. Check to see if free from calcium.

Comment is solicited concerning the method or any modifications that might well be adopted.

COLLABORATORS

The following collaborators reported:

Henry C. Fuller, Washington, D. C.
Carl S. Ferguson, Division of Food and Drugs, Boston, Mass.
R. J. Hennessy, Office of State Food Commissioner and Chemist, Bismarck, N. D.
Elmer W. Campbell.

The results are shown in the table.

Work should be undertaken on the following subjects: Slenderizers, hair dyes, hair waves, lipsticks, rouges, eyebrows, eyelashes, shaving creams, tooth pastes and powders, face creams, face lotions, perfumes and toilet waters, bath salts, hair pomades, hair anticrinkle, hair removers, hair bleaches, B. O. preparations, hair tonics, and face powders.

It is recommended that study be continued on the soluble barium content of the colors used in cosmetics as well as in finished products and that the studies be enlarged to include arsenic determinations.

REPORT ON CEREALS

By V. E. MUNSEY (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

Methods of Analysis, A.O.A.C. contains a chapter on cereal foods that has numerous methods under the headings of wheat flour, bread, baked products other than bread, and macaroni products. With few exceptions, the methods under wheat flour were adopted after studies had been made on this product. Other cereals, such as whole wheat flour, gluten flour, corn meal, rye flour, buckwheat flour, malt and barley flour, rice flour, rolled oats, oat meal, and oat flour may be analyzed by similar procedures. However, before the appropriate methods under wheat flour are referred to as applicable to these other cereal products some confirmatory studies should be made. Likewise, under the heading "baked products other than bread" methods are given for total solids and crude fiber. It is probable that these methods would also apply to baked products other than bread, but this should be determined by experimental study on such products as crackers, cookies, and cakes.

It is recommended[1]—

(1) That an associate referee be appointed to study the application of the methods under wheat flour for the determination of water, ash, protein, fat, and crude fiber to corn meal, corn flour, and corn starch.

(2) Likewise for rolled oats, oat meal, and oat flour.

(3) Likewise for rye flour and buckwheat flour.

(4) Likewise for malt barley, barley flour, and rice flour.

(5) That an associate referee be appointed to study the application of methods under bread for the determination of ash, fat and, protein to such baked products as crackers, cookies, and cakes.

(6) That the tentative method for the preparation of sample of bread, *Methods of Analysis, A.O.A.C.*, 1935, 221, **50**, be made official (final action).

(7) That the method for collection and preparation of sample in macaroni products, *Ibid.*, 228, **68**, be made official (final action).

(8) That the method for the determination of apparent viscosity of flour, *This Journal*, **20**, 380, be adopted as official (final action). Through error last year the reference was given as *Methods of Analysis, A.O.A.C.*, 1935, 221.

(9) That the magnesium acetate method, *This Journal*, **20**, 69, be made official (final action).

(10) That an attempt be made to develop a method for determining sodium chloride-free ash.

(11) That the associate referee continue his studies on H-ion concentration of flour.

(12) That the search for a rapid and accurate method for starch be continued.

(13) That further study be given to the improvement of the polarimetric methods for starch.

(14) That the A.O.A.C. tentative method for the determination of acidity in flour, 208, **13**, be dropped.

(15) That the method proposed by the associate referee for determination of fat-acidity of flour be adopted as tentative.

(16) That further study be made of the proposed methods for acidity in flour and other cereal products.

(17) That the collaborative study of the proposed method for determination of reducing and non-reducing sugars in flour be continued.

(18) That the associate referee continue his studies on the baking test for soft wheat flour.

(19) That the study of methods for the determination of chlorine in bleached flour fat be continued.

(20) That the method for the measurement of carotenoid pigments in flour, *This Journal* 21, 339, be studied next year with a view to substituting

water-saturated normal butyl alcohol for the Varsol-alcohol mixture.

(21) That study of the method for the determination of benzoyl peroxide in flour be continued.

(22) That collaborative study on determination of the carbon dioxide content of self-rising flour be continued.

(23) That the citric acid procedure for calculation of milk solids be not further studied collaboratively for the present.

(24) That the lactose procedure be more extensively studied collaboratively.

(25) That the so-called fat method be not further studied collaboratively in the present form.

(26) That study be made of a method for estimation of butterfat content based on the direct saponification and distillation of bread samples without extraction of the fat.

(27) That the tentative method for extract soluble in cold water, 213, 30, be adopted in its modified form, *This Journal*, 22, 76, as official (first action).

(28) That collaborative study be continued on extract soluble in cold water on flour and other cereals.

(29) That the associate referee continue the study of ergot in flour.

(30) That the study on proteolytic enzymes in flour be continued.

(31) That further collaborative work on measurement of flour and bread color by the N. A. colorimeter be dropped.

(32) That study be continued on the sampling and preparation of flour and bread for color measurements by the photoelectric cell method based on reflectance.

(33) That work be continued on the determination of soybean flour in cereal products.

(34) That the study on macaroni be continued on the basis of the work of the present associate referee, but that no new associate referee be appointed this year to fill the vacancy caused by the inability of the present referee to serve any longer.

(35) That the associate referee continue his studies on the methods of determining cellulose as an index of the whole wheat content of cereal products.

(36) That further study be made of methods for determining the ash of the original flour in phosphated and self-rising flours, especially of old self-rising flours, with special reference to—

   (a) Analysis for sodium chloride content of self-rising flours and the ash of the extracted flours.
   (b) The study of means other than extraction with carbon tetrachloride for 100 per cent separation of the sodium chloride from old self-rising flours.

REPORT ON ASH IN FLOUR, MACARONI PRODUCTS, AND BAKED PRODUCTS

By L. H. BAILEY (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

At the 1937 meeting of the A.O.A.C. the magnesium acetate method was made official (first action) as an alternative and rapid method of determining ash in flour, macaroni products, and baked products.

In order to obtain further collaborative work on this method, the Associate Referee submitted samples of whole wheat flour, corn meal, and bread to several analysts. They were furnished copies of the magnesium acetate method and were requested to determine ash on the samples submitted by the proposed and the present official A.O.A.C. methods. The following collaborators reported: V. E. Munsey, M. H. Neustadt, J. T. Keister, B. L. Kaspin, and L. H. Bailey.

The results are shown in the table.

Collaborative ash results (per cent)

COLLABORATOR	1		2		3		4		5	
	A.O.A.C.	Mg. Ao₂	A.O.A.C.	Mg. Ao₂	A.O.A.C.	Mg. Ao₂	A.O.A.C.	Mg. Ac₂	A.O.A.C.	Mg. Ao₂
Corn Meal	1.28	1.31	1.34	1.33	1.22	1.30	1.33	1.33	1.31	1.30
	1.27	1.31	1.34	1.35	1.23	1.35	1.34	1.33	1.33	1.28
	1.29	1.36		1.29	1.32		1.33	1.34	1.35	1.33
	1.31	1.36		1.32					1.32	1.32
Mean	1.29	1.34	1.34	1.32	1.26	1.33	1.33	1.33	1.33	1.31
Whole	1.83	1.87	1.81	1.85	1.84	1.85	1.89	1.84	1.84	1.85
Wheat	1.82	1.87	1.82	1.84	1.92	1.92	1.83	1.82	1.85	1.84
Flour	1.82	1.89		1.79			1.85	1.82	1.88	1.86
	1.83	1.90		1.82					1.88	1.88
Mean	1.83	1.88	1.82	1.83	1.88	1.88	1.86	1.83	1.86	1.86
Bread	1.81	1.75	1.79	1.66	1.56	1.60	1.91	1.75	1.82	1.75
	1.80	1.76	1.82	1.60	1.54	1.69	1.90	1.78	1.76	1.76
	1.86	1.85		1.62			1.91	1.76	1.73	1.66
	1.87	1.87		1.66					1.72	1.68
Mean	1.84	1.81	1.81	1.64	1.56	1.64	1.91	1.76	1.76	1.71

The collaborators, for the most part, obtained close agreement, both individually and with each other.

The values for corn meal and whole wheat flour by the magnesium acetate method agree well with those by the present A.O.A.C. method. With the bread there was more difficulty as was to be expected owing to

the added sodium chloride present and the greater losses that occur in ashing at 700° C., as required by the rapid method, than at 550° C. (A.O.A.C. method). This fact should be taken into account with any sample containing an appreciable quantity of sodium chloride.

There is need for a method for the determination of sodium chloride in cereal products.

It is recommended*—

(1) That the magnesium acetate method be made official (final action).

(2) That an attempt be made to develop a method for obtaining a sodium chloride-free ash.

No report on H-ion concentration of flour was given by the Associate Referee.

REPORT ON STARCH IN FLOUR

By C. Y. HOPKINS (National Research Council,
Ottawa, Canada), *Associate Referee*

It has been demonstrated by the earlier work of the Association that starch in flour may be determined conveniently by the modified Rask method, which is now tentative.

Last year's collaborative study indicated that the method was unsuited to the analysis of certain cooked products, and it was recommended that work should be directed towards the selection of a new method that would be applicable to all cereal products, cooked and uncooked.

A review of all available methods was made, including several appearing in the recent literature, and it seemed that none was sufficiently promising to submit for collaborative study without preliminary investigation.

CONSIDERATION OF AVAILABLE METHODS

A number of the methods found in the literature were developed for the analysis of plant material of low starch content and, for one reason or another, do not appear to be applicable to the problem at hand. These include the methods of Denny,[1] Hanes,[2] Pucher and Vickery,[3] and Sullivan.[4]

The use of diastase (Denny, Hanes) is open to question since Sullivan has shown that diastase preparations are not entirely specific for starch.[4]

The method of Pucher and Vickery is colorimetric and requires the use of a spectrophotometer, an instrument that is not available in all laboratories.

* For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).
1 Contrib. Boyce Thompson Inst., 6, 129 (1934).
2 *Biochem. J.*, 30, 168 (1936).
3 *Ind. Eng. Chem. Anal. Ed.*, 8, 92 (1936)
4 *This Journal*, 18, 621 (1936).

The Sullivan method has been carefully worked out and is capable of giving very accurate results. It was studied by the Associate Referee in 1936 and was found to be quite lengthy and to require extensive manipulation. These objections apply also to the other methods listed.

The Mannich and Lenz polarimetric method[1] was studied in 1936. It was found to give fairly good results with wheat flour but was subject to some manipulative difficulties.

Another polarimetric method was brought to the attention of the Associate Referee this year. It is a modification of the Lintner-Schwarz method[2] and was used with considerable success in determining starch in barley.

The method of Chinoy et al.,[3] in which the starch is weighed as starch iodide, was tried in 1935 with little success.

COLLABORATIVE WORK

Several of the proposed methods were tried out by apportioning them among the collaborators. The results are given in Table 1.

TABLE 1.—*Collaborative results*

ANALYST	MATERIAL	METHOD	PER CENT STARCH	MEAN
1	Cottonseed meal	Mannich-Lenz	negative rotation	
6	Cottonseed meal	Mannich-Lenz	negative rotation	
2	Cottonseed meal	Lintner-Schwarz		3.44
5	Cottonseed meal	Lintner-Schwarz	3 62, 3.75, 3.62	3.66
2	Cottonseed meal	Acid hydrolysis		3.25
4	Cottonseed meal	Rask	1.09, 1.28	1.18
6	Wheat starch	Mannich-Lenz		87.9
2	Wheat starch	Lintner-Schwarz	82.0, 81.9, 80.7	81.5
5	Wheat starch	Lintner-Schwarz	72.37, 72.50	
			72.25, 72.50	72.40
2	Wheat starch	Acid hydrolysis	83.22, 82.68	82.95
6	Wheat starch	Chinoy	would not filter	
2	Food product	Lintner-Schwarz		11.44
2	Food product	Acid hydrolysis		11.22

The cottonseed meal was ground to minus 100 mesh and when boiled with aqueous calcium chloride the solution gave a faint color with iodine, showing that it contained a mere trace of starch.

The wheat starch contained 10.8 per cent moisture and the sum of the impurities (protein, sugars, ash, fat, fiber) was 1.0 per cent. The starch content is therefore assumed to be 88.2 per cent.

Analysts 1 and 6 reported a negative rotation when analyzing cottonseed meal by the Mannich-Lenz polarimetric method. This is probably due to protein material that was not eliminated in the procedure. Re-

[1] *Can. J. Research*, 11, 751 (1934).
[2] *Z. Brauwesen*, 1913, No. 7 and 8.
[3] *Analyst*, 59, 673 (1934).

peating the work and adding phosphotungstic acid to precipitate pro-
teins, Analyst 6 was able to reduce the observed rotation from −0.10°
to −0.01°. The latter figure is within experimental error.

In addition to the collaborative work, analyses of potato starch, potato
flour, and barley kernels were carried out by two other chemists. They
used the Mannich-Lenz method with fair success, although some difficulty
was reported due to frothing. Some of the results with barley are shown
in Table 2.

TABLE 2.— *Starch in barley*

MATERIAL	MANNICH-LENZ METHOD	LINTNER-SCHWARZ METHOD
	per cent	*per cent*
High protein barley (1)	53.7, 53.1	52 6, 52.8
High protein barley (2)	50.2, 50.8	50.1, 51.5
Low protein barley (3)	58.4, 58.9	60.0, 59.8
Low protein barley (4)	58.2, 58.2	58.9, 59.0

DISCUSSION

The results with cottonseed meal indicate that those methods using
acid to disperse the starch tend to give high results owing to the inter-
ference of hemicelluloses. The Mannich-Lenz method (using calcium
chloride) gave a low result due to the interference of proteins. A modi-
fication of the method is suggested.

In the analysis of wheat starch, Analyst 2 obtained fairly good agree-
ment between the Lintner-Schwarz method and direct acid hydrolysis.
The Mannich-Lenz method gave a higher figure. Analyst 5 obtained
excellent checks using the Lintner-Schwarz method but was not in agree-
ment with Analyst 2. The Chinoy method was again found to be un-
satisfactory.

The following comments were made:

Mannich-Lenz method.—Less tedious than other methods. Frothing is difficult to
control.

Lintner-Schwarz method.—This method has some very good features. Ten to
twelve analyses can be done in one day.

Pucher-Vickery method.—Undoubtedly lengthy. May not be applicable to sam-
ples high in starch. The blue color with iodine is not always specific for starch.

The possibility of determining starch by fermentation to alcohol was
considered by one collaborator. He reported that the results would not
be sufficiently quantitative, even under carefully controlled conditions.

MODIFIED LINTNER-SCHWARZ METHOD

Weigh the sample (2.0–2.5 grams) into a 50 cc. round-bottomed centrifuge tube
with lip. Wash by adding 10 cc. of aqueous alcohol (D_{20}, 0.88) and stirring thoroly
with a glass rod. Place the rod carefully aside while the mixture is centrifuged and
the liquid poured off. Repeat the washing until 60 cc. of wash liquid has been used,

stirring each time with the same rod. (For samples containing more than 2–3% of fat, a preliminary wash with ether is advised.)

Wash the residue into a 100 cc. volumetric flask by adding 50 cc. of H_2SO_4, sp. gr. 1.40, in portions. Place the mixture in a thermostat at 20° C. for 1 hour and shake occasionally. Add 10 cc. of freshly prepared 2% phosphotungstic acid solution and fill the flask to the mark.

A quantity of the liquid is filtered, the first 10 cc. is discarded, and the filtrate is polarized. $\alpha D = 200$.

RECOMMENDATIONS*

It is recommended—

(1) That the search for a rapid and accurate method for starch be continued.

(2) That further study be given to improvement of the polarimetric methods.

REPORT ON ACIDITY IN FLOUR

By Lawrence Zeleny (Agricultural Marketing Service, Washington, D. C.), *Associate Referee*

Titratable acidity has frequently been used as a measure of the age or "condition" of a flour, because the acidity increases with age under normal storage conditions and in general increases more rapidly under storage conditions that favor deterioration.

The methods most commonly used for determining titratable acidity in flour may be summarized as follows:

(1) *A.O.A.C. tentative method.*[1]—This method specifies digesting of the flour for one hour at 40° C., titrating of the filtered extract with standard alkali, and the use of phenolphthalein as an indicator. Acidity is reported as per cent lactic acid.

(2) *Balland or Greek method.*[2]—The Greek government has adopted as its official method for determining the acidity of flour a method by which the flour is extracted with 85 per cent alcohol and filtered and the filtrate is titrated with alcoholic potash, curcuma being used as an indicator. Results are expressed as per cent sulfuric acid.

(3) *Schulerud's method.*[3]—The flour is digested with 67 per cent alcohol, and the filtrate is titrated with standard alkali, phenolphthalein being used as an indicator. Results are expressed as the number of milliliters of normal alkali required to neutralize the acid from 100 grams of flour.

All acidities determined by the above methods are expressed in different terms, and even when these values are converted to the same

* For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).
[1] *Methods of Analysis, A.O.A.C.*, 1935, 208.
[2] Am. Assoc. Cereal Chemists, Cereal Laboratory Methods, 1935, 41–42.
[3] *Ibid.*, 42.

terms, wide differences are shown. A high acidity by one method is generally associated with high acidities by the other methods, but no mathematical relationship exists between values obtained by the different methods, indicating in each case a different type of acid fraction.

SEPARATION OF ACIDS INTO CLASSES

The acidic substances present in flour consist principally of three classes of compounds: (1) acid phosphates, (2) amino acids, and (3) free fatty acids. These three types of acids are present as normal constituents of all wheat and flour, and under certain conditions they may be increased in quantity by the enzymatic hydrolysis of phytin, protein, and fat, respectively.

The acid phosphates and amino acids in flour are soluble in water and dilute ethyl alcohol, but relatively insoluble in strong alcohol. Most of the amino acids, however, are neutral in reaction in water and dilute alcohol and therefore do not contribute to the titratable acidity of water extracts even though present. In 85 per cent alcohol, on the other hand, the carboxyl groups of most of the amino acids may be titrated quantitatively.

The free fatty acids in flour are essentially insoluble in water but are soluble in fat solvents or in strong alcohol.

In order to determine the relative amounts of the various types of acidic substances extracted by different concentrations of alcohol, the following experiment was performed:

Eleven 10 gram portions of a commercial hard red winter wheat flour were shaken for 30 minutes with 100 cc. portions of neutral alcohol-water mixtures of different concentrations. The suspensions were then centrifuged, and two 25 cc. aliquots of each centrifugate were taken. To one of these aliquots was added sufficient neutral 95 per cent alcohol to bring the concentration of alcohol in the mixture to 85 per cent. To the other aliquot was added sufficient CO_2-free water to reduce the concentration of alcohol 5 per cent. The titration value of each solution was determined with phenolphthalein as an indicator.

A second series of eleven 10 gram portions of the same flour was extracted for 16 hours with petroleum ether in Soxhlet extractors. The free fatty-acid content of the extracts was determined, the residues were extracted with various concentrations of alcohol, and the acidities of the extracts were determined in the same manner as in the case of the original flour samples.

From the data obtained it is possible to divide the acidity extracted from the original flour by each of the 11 concentrations of alcohol into three distinct fractions, the amino-acid acidity, the acid phosphate acidity, and the fat acidity (Table 1). This may be understood more clearly by referring to the curves in Figure 1. Curve I represents the total titratable

acidity of each extract as determined by titrating the extracts of the original flour in 85 per cent alcohol. Curve II represents similar values for the alcoholic extracts of the fat-free flour, and therefore represents total acidity minus fat acidity. Obviously the differences between the values in Curve I and Curve II represent the fat acidity, which is plotted as

TABLE 1.—*Different acid fractions extracted from a commercial hard red winter wheat flour by different concentrations of alcohol*

(Acidity values are in terms of mg. of KOH required to neutralize acid extracted from 100 grams of flour, dry-matter basis.)

| CONCENTRA-TION OF ALCOHOL | ORIGINAL FLOUR | | PETROLEUM ETHER EXTRACTED FLOUR | | AMINO-ACID ACIDITY C-D | FAT ACIDITY* A-C |
	A ACIDITY IN 85% ALCOHOL. TOTAL ACIDITY	B ACIDITY IN 5% ALCOHOL	C ACIDITY IN 85% ALCOHOL	D ACIDITY IN 5% ALCOHOL-"PHOSPHATE" ACIDITY		
per cent						
95	47.6	29.6	28.2	12.6	15.6	19.4
90	57.0	34.1	39.1	18.9	20.2	17.9
80	109.5	57.5	91.6	41.8	49.8	17.9
70	135.6	65.5	117.6	54.3	63.3	18.0
65	144.1	66.9	126.6	60.6	66.0	17.5
60	143.7	68.7	129.7	60.2	69.5	13.5
50	140.1	66.4	131.5	60.6	70.9	8.6
40	134.7	67.3	131.1	61.5	69.6	3.6
30	132.4	58.8	129.7	55.7	74.0	2.7
20	127.0	56.6	126.2	55.2	71.0	0.8
10	141.0	65.1	140.5	65.1	75.4	0.5
0	153.1	73.2	154.4	70.9	83.5	−1.3

* Fat acidity as determined by petroleum ether extraction: 19.0.

Curve VI. Curve V represents the acidity of the alcoholic extracts of the fat-free flour as determined by titration in 5 per cent alcohol and may be considered to be essentially the curve for the acid phosphates. The difference between Curve II and Curve V represents the amino acid acidity, which is plotted separately as Curve IV.*

The high values for amino-acid acidity, phosphate acidity, and total acidity in the water (0 per cent alcohol) and 10 per cent alcohol extracts are probably due to the presence of acids formed by enzymatic action during the extraction process, in addition to the acids already present in the flour. That this is the case is evidenced by the fact that in water solu-

* It should be pointed out that the acid fraction which is designated "phosphate" acidity and which is represented by Curve V in Figure 1 consists of certain other acid-reacting substances in addition to the acid phosphates. These additional acidic factors consist of one of the carboxyl groups of any dicarboxylic amino acids present, the acid-binding power of any peptized protein present, and possible traces of organic acids not soluble in petroleum ether. Acid phosphates, however, undoubtedly account for the major part of this fraction.

It should further be pointed out that the amino-acid fraction as represented by Curve V, since it is based on the Foreman titration, does not account quantitatively for any proline or dibasic amino acids that may be present.
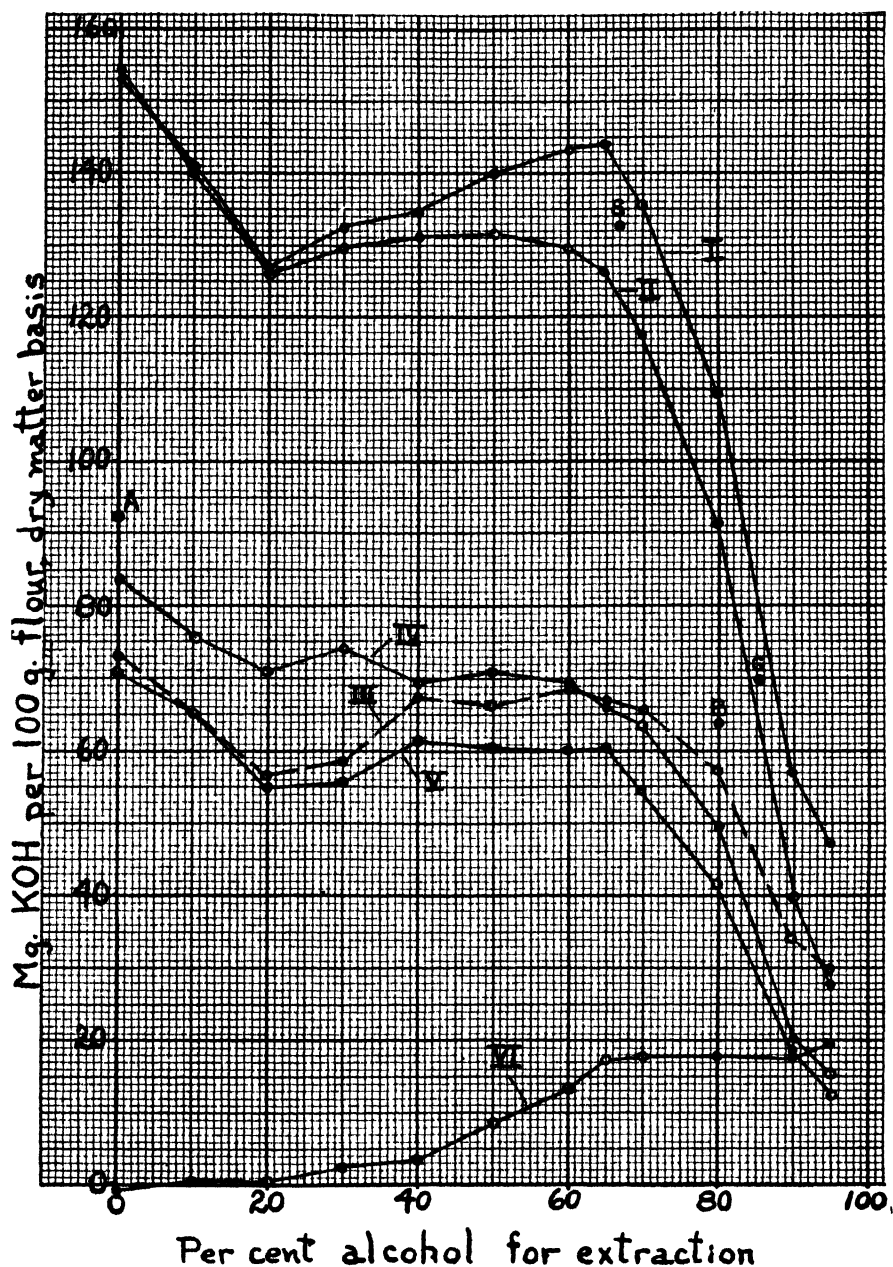
FIG. 1.—ACID FRACTIONS EXTRACTED FROM A COMMERCIAL HARD RED WINTER WHEAT
FLOUR BY DIFFERENT CONCENTRATIONS OF ETHYL ALCOHOL.

Curve I. Total acidity. Titration in 85 per cent alcoholic solution. Curve II. Total acidity of fat-free
sample. Titration in 85 per cent alcoholic solution. Curve III. Acidity by titration in 5 per cent alcoholic
solution. Curve IV. Amino acidity. II minus V. Curve V. "Phosphate" acidity. Titration of extract of fat-
free sample in 5 per cent alcoholic solution. Curve VI. Fat acidity. I minus II. Point A. Acidity by the
A.O.A.C. tentative method. Point G. Acidity by the Greek or Balland method. Point S. Acidity by Schule-
rud's method.

tion the quantity of acid extracted is a function of the time and temperature of extraction, whereas in alcoholic solutions stronger than 20 per cent no appreciable increase in acidity occurs after the first 15 minutes of digestion. Thus the present A. O. A. C. tentative method must be considered empirical, as the acid determined is the sum of the acid extracted plus the acid formed by enzymatic action under the conditions of extraction specified by the method.

If the extracts made with alcohol concentrations of 20 per cent or over, in which enzymatic activity appears to be inhibited, are considered, it will be observed that a maximum total acidity is obtained at an alcohol concentration of about 65 per cent, thus essentially confirming Schulerud's observation that 67 per cent alcohol extracts the maximum quantity of acid from flour. It should be noted, however, that whereas Schulerud's 67 per cent alcohol extract may contain the maximum total acidity, this concentration of alcohol is too low to determine the maximum acidity. The strength of alcohol in such an extract should be increased to 85 per cent before titration in order to account for the maximum possible amino acid content.

It will also be observed that the amino-acid content is essentially constant at a maximum value in extracts containing between 20 per cent and 60 per cent alcohol, and "phosphate" acidity between 20 per cent and 65 per cent alcohol. Fat acidity values are nearly constant at concentrations of alcohol greater than 65 per cent and agree very well with the value determined by petroleum ether extraction. Thus no single concentration of alcohol is capable of extracting quantitatively all three types of acidic products, when the ratio of solvent to flour commonly employed in acidity determinations is used. Alcohol concentrations between 60 per cent and 70 per cent will in general extract most of the acids of all three types from flours containing less than 1 per cent of fat.

Curves similar to those shown in Figure 1 are obtained for both wheat and corn when maximum total acidity values in all samples tested are extracted with alcohol of between 60 per cent and 70 per cent.

The following procedure, based on the foregoing experiments, has been used for determining the three classes of acid-reacting compounds in flour:

METHOD

Extract 10 gram portions of the flour for 16 hours with petroleum ether in Soxhlet extractors. After evaporation of the solvent, dissolve the extracts in 50 cc. of 1:1 benzene-ethyl alcohol mixture containing 0.02% phenolphthalein, and determine fat acidity by titration with standard alkali. Subtract a blank titration on the benzene-alcohol mixture from the titration values of the extracts.

Suspend the extracted flours in 100 cc. of 60% ethyl alcohol neutral to phenolphthalein in stoppered flasks. Shake the suspensions at frequent intervals for 30 minutes and then filter. Dilute one 25 cc. aliquot of the filtrate with neutral 95% alcohol to a concentration of 85% alcohol and titrate. Dilute a second 25 cc. aliquot with CO_2-free water to a concentration of 5% alcohol and titrate. (The titration in

5% alcohol corresponds to the "phosphate" acidity and the difference between the two titrations corresponds to the amino-acid acidity.)* Calculate the results as the number of mg. of KOH required to neutralize the acids extracted from 100 grams of the flour on a dry-matter basis.

SIGNIFICANCE OF DIFFERENT TYPES OF ACIDITY

Zeleny and Coleman[1] showed that as wheat and corn deteriorate in storage the fat acidity increases rapidly even during the early incipient stages of deterioration, whereas the phosphate acidity and amino acid acidity increase only during relatively advanced stages of deterioration.

TABLE 2.—*Acidity of flour at intervals during storage at 32° C.*

SAMPLE NUMBER	CLASS OF WHEAT	FAT ACIDITY FLOUR, DAYS AT 32° C.				PHOSPHATE ACIDITY FLOUR, DAYS AT 32° C.				AMINO ACID ACIDITY FLOUR, DAYS AT 32° C.			
		0	20	112	203	0	20	112	203	0	20	112	203
1	Soft Red Winter	17	24	46	60	40	42	43	48	69	72	67	70
2	Soft Red Winter	16	25	50	61	42	44	45	51	63	72	67	67
3	Soft Red Winter	18	24	49	61	42	48	46	47	69	71	67	68
4	Soft Red Winter	18	25	52	65	38	40	45	48	68	72	65	64
5	Soft Red Winter	18	26	46	58	40	43	43	47	63	69	65	62
6	Soft Red Winter	17	27	50	61	49	46	46	49	68	74	72	67
7	Soft Red Winter	19	26	49	61	44	48	45	54	69	73	70	67
8	Soft Red Winter	20	31	60	75	40	45	46	49	74	75	73	69
9	Soft Red Winter	24	30	54	65	39	41	45	48	66	73	65	67
10	Soft Red Winter	22	33	60	74	37	48	46	50	68	73	74	69
11	Soft Red Winter	26	36	55	72	43	56	55	59	69	76	75	68
12	Soft Red Winter	25	39	74	87	50	55	57	59	81	81	80	77
Av.	Soft Red Winter	20	29	54	67	42	46	47	51	69	73	70	68
13	Hard Red Spring	17	21	40	51	47	56	59	64	89	113	89	87
14	Hard Red Spring	22	44	90	100	48	58	65	81	117	116	120	115
15	Hard Red Spring	19	31	69	89	56	64	68	70	103	100	105	108
Av.	Hard Red Spring	19	32	66	80	50	59	64	72	103	110	105	103
16	Hard Red Winter	20	34	71	100	46	50	50	57	72	78	80	76
17	Hard Red Winter	17	27	58	71	41	47	50	52	82	85	79	79
Av.	Hard Red Winter	18	30	64	85	43	48	50	54	77	81	79	77
Av.	All Samples	20	30	57	71	44	49	50	55	76	81	77	75

The same type of study was applied to flour in storage. Seventeen samples of freshly milled straight grade unbleached flour were stored at 32° C. at moisture contents between 12.0 and 12.3 per cent, and the different types of acidity were determined at intervals over a period of 203

* See footnote on p. 528.
[1] *Cereal Chem.*, 15, 580–95 (1938).

days. The results are given in Table 2, and the average percentages of increase in the different types of acidity are shown graphically in Figure 2. Here again the rate of increase in fat acidity is much greater than that of the other types of acidity, and fat acidity alone showed a large and easily measurable increase during the first 20 days of storage.

Kozmin[1] and more recently Barton-Wright[2] showed that deterioration of gluten quality during storage of flour is due largely to the accumulation of free fatty acids, particularly the unsaturated fatty acids. Since the extent of deterioration is roughly proportional to the concentration of
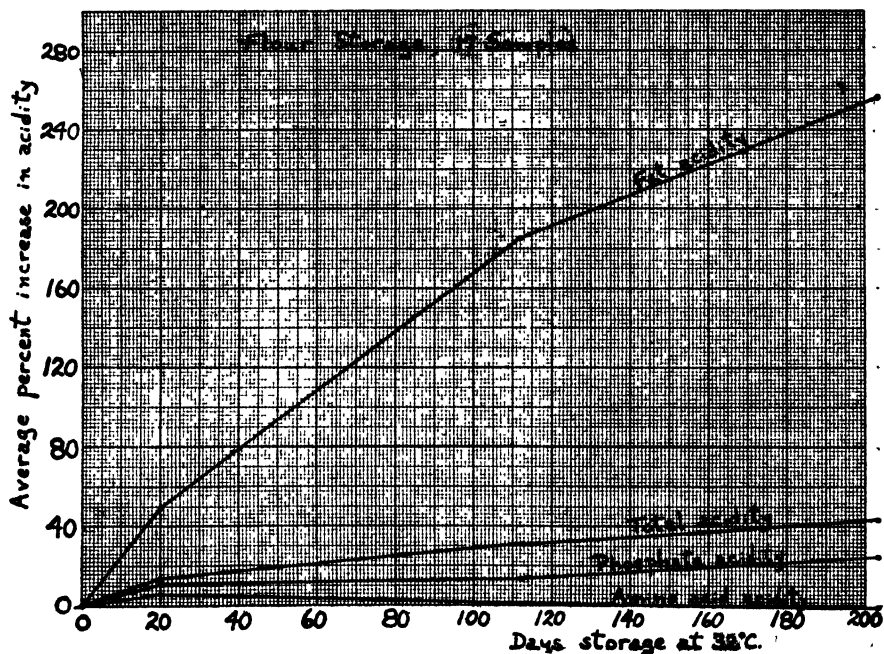
FIG. 2.—AVERAGE PERCENTAGE INCREASE IN ACIDITY OF DIFFERENT TYPES IN 5 SAMPLES OF FLOUR STORED AT 30°C. AND AT MOISTURE CONTENTS BETWEEN 12.0 AND 12.3 PER CENT.

free fatty acids in the flour, the "fat acidity" value as herein defined should be a better measure of such deterioration than would the acid value of the flour fat.

Baking tests with a basic formula were made at intervals on three samples of Hard Red Spring flour and two samples of Hard Red Winter flour during storage, and loaf volumes were compared with fat acidity values. Figure 3 shows graphically the decrease in loaf volume associated with increase in fat acidity for each of these flours. The winter wheat

[1] *Cereal Chem.*, 12, 165–171 (1935).
[2] *Ibid.*, 15, 521–540 (1938).

flour appears to be somewhat more resistant to the effects of high fat acidity than do the spring wheat flours, but in all cases high fat acidity was associated with significant decreases in loaf volume.
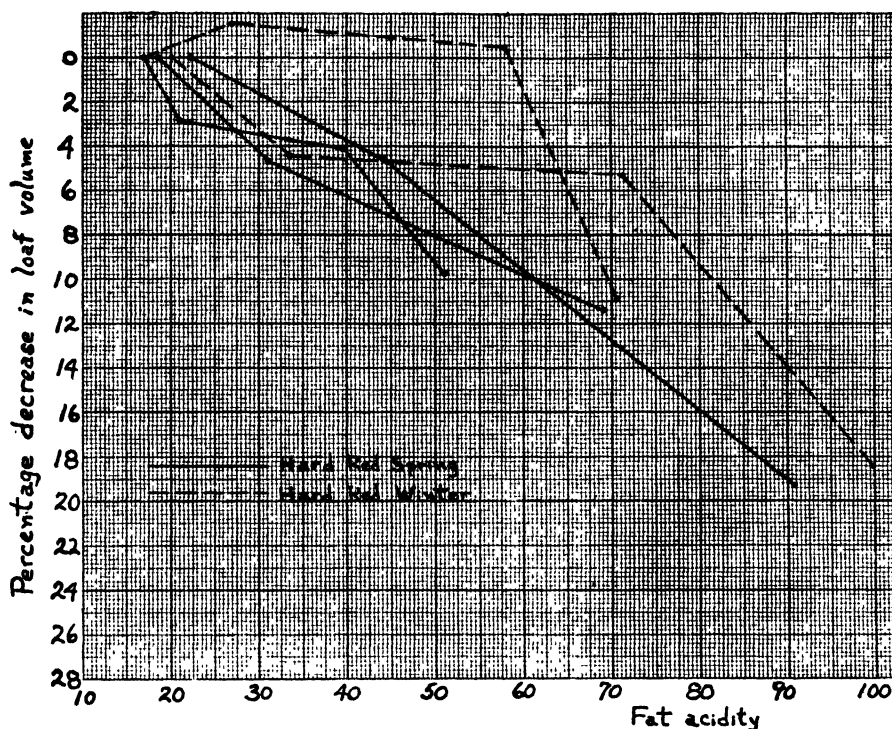
FIG. 3.—PERCENTAGE DECREASE IN LOAF VOLUME OF BREAD ASSOCIATED WITH INCREASE IN FAT ACIDITY OF FLOUR.

DISCUSSION

None of the three principal methods used for determining titratable acidity in flour appears to be based on a fundamental concept of the nature of the acids being determined.

The A.O.A.C. tentative method determines acidity due primarily to acid phosphates. The amino acids are present in the extract but are not determined by the titration procedure used. Fatty acids are neither extracted nor determined. The principal weakness of the method appears to be that it determines not only the acid phosphates originally present in the flour but also acid phosphates formed by enzymatic action during the extraction process.

The Greek or Balland method extracts and determines essentially all the free fatty acids plus varying fractions (generally less than half) of the acid phosphates and amino acids.

Schulerud's 67 per cent alcohol method extracts the major part of all three types of acids but fails to determine quantitatively the amino acids extracted.

Fat acidity appears to be a better index of the aging of flour than are either of the other principal types of acidity present or than any combination of the various types. A certain amount of evidence exists indicating that the accumulation of free fatty acids in flour is largely responsible for the deterioration of its baking quality. Whether or not this view can be fully substantiated, the fact remains that deterioration in storage is accompanied by a large and easily determined increase in fat acidity.

COLLABORATIVE STUDY

Four samples of flour of different ages were analyzed for fat acidity by six collaborators using the following method:

Extract duplicate 10 gram samples with petroleum ether for approximately 16 hours, using a Soxhlet, or similar extraction apparatus, and double thickness paper thimbles or Alundum R.A. 360 thimbles.

Completely remove the solvent from the extract by evaporation on the steam bath and dissolve the extract in the extraction flask with 50 cc. of a mixture of equal parts by volume of ethyl alcohol and benzene, containing approximately 0.02% of phenolphthalein.

Titrate the dissolved extract with carbonate-free standard alkali to a distinct pink color. For convenience use exactly 0.0178 N alkali for the titration in order to simplify calculations, 1 cc. of this solution being equivalent to 1 mg. of KOH. Make a blank titration on 50 cc. of the benzene-alcohol mixture and subtract the value obtained from the titration value of the sample.

Report fat acidity as the number of mg. of KOH required to neutralize the free fatty acids from 100 grams of flour on a dry-matter basis.

The results obtained by the various collaborators are listed in Table 3.

TABLE 3.—*Fat acidity values obtained by seven collaborators* on four samples of flour (Results are averages of duplicate determinations and are expressed in terms of mg. of KOH required to neutralize free fatty acids in 100 grams of flour on a dry-matter basis.)

SAMPLE NUMBER	COLLABORATORS							AVERAGE	MEAN DEVIATION
	A	B	C	D	E	F	G		
1	29.4	26.0	28.9	28.2	24.3	28.7	27.2	27.5	1.5
2	69.0	66.3	75.7	61.5	64.2	68.2	58.6	66.2	4.1
3	65.8	64.5	73.5	62.5	65.1	62.1	60.8	64.9	2.8
4	20.8	18.3	26.9	18.1	28.9	21.5	18.2	21.8	3.5

* The collaborators participating in this work are listed as follows: L. H. Bailey, Bureau of Chemistry and Soils, Washington, D. C. R. A. Barackman, Victor Chemical Works, Chicago Heights, Ill. Leo Kreno-wits, Bureau of Agricultural Economics, Washington, D. C. M. H. Neustadt, Bureau of Agricultural Economics, Washington, D. C. H. K. Parker, Wallace & Tiernan Co., Inc., Newark, N. J. Julius Siebenberg, Schwartz Laboratories, Inc., New York, N.Y. The Associate Referee. These names are not listed in the order used in Table 3.

RECOMMENDATIONS*

It is recommended—

(1) That the A.O.A.C. tentative method for acidity in flour be dropped.

(2) That the proposed method for determining the fat acidity of flour be adopted as tentative.

(3) That further study be made of the proposed methods for acidity in flour and other cereals.

REPORT ON SUGAR IN FLOUR

By R. M. SANDSTEDT (University of Nebraska, Lincoln, Neb.),
Associate Referee

The identity of the reducing and non-reducing sugars of flour is still questionable. Genevois and Pavloff,[1] Geoffroy,[2] Colin and Belval[3,4,5,6,7] and Guillement[8] believe that the reducing sugars are hexoses and that much of the non-reducing sugar is levosin. Levosin is a levulosan that is fermentable in dough or in the presence of the fermentation activators that occur naturally in flour.[2,5,7,8] This levulosan is probably much more abundant in flours produced from European wheat than in those produced from American hard wheats.[1] Accordingly, until the identity of these sugars is more completely known, it is advisable to use the terms reducing sugars and non-reducing sugars rather than the specific terms maltose and sucrose. Since levosin is non-reducing, soluble in water, easily hydrolyzed, and is fermentable in dough, for practical purposes it may be included with the non-reducing sugars and calculated as sucrose.

The tentative method for reducing sugars and sucrose in flour as given in *Methods of Analysis, A.O.A.C.*, 1935, is the same as that given for the determination of sugars in feeds. This method involves the extraction of the sugars with 50 per cent alcohol and the subsequent clarification with lead acetate. This procedure is not satisfactory with flour owing to the solubility of gliadin in 50 per cent alcohol and to the poor clarification obtained with lead acetate on flour extract.[9] Since water extraction with subsequent acid tungstate clarification has proved satisfactory for determination of the maltose value[10,11] of flour, it would seem to be the logical method for the extraction and clarification of the other sugars.

* For report of Subcommittee D and action by the Association, see *This Journal* 22, 68 (1939).
[1] *Comp. rend.*, 200, 690 (1935).
[2] *Bull. soc. chim. biol.*, 17, 848 (1935).
[3] *Comp. rend.*, 200, 2032 (1935).
[4] 14 me. Congr. chim. ind., Paris. Oct. 1934.
[5] *Bull. soc. chim. biol.*, 17, 1040 (1935).
[6] *Bull. soc. chim.*, 2, 1907 (1935).
[7] *Bull. soc. chim. biol.*, 19, 65 (1937).
[8] *Comp. rend.*, 201, 1517 (1936).
[9] Blish, *J. Biol. Chem.*, 33, 551 (1918).
[10] Rumsey, *Am. Inst. Baking Bull.*, 8 (1922).
[11] Blish, *This Journal*, 16, 497 (1933); Blish and Sandstedt, *Cereal Chem.*, 10, 189 (1933).

Sandstedt[1] has proposed a method for the determination of sucrose directly from the extract that is obtained for the determination of maltose value. He utilizes the acid added for clarification for the sucrose hydrolysis. It would seem that this method for sucrose could also be used on the extract made for the determination of the original reducing sugars before diastasis.[11] Thus the non-reducing sugars could be determined in conjunction with either the maltose value or the reducing sugars.

Accordingly, a method for reducing and non-reducing sugars (based on the ferricyanide method for non-reducing sugars[11]) was submitted to collaborative study. The collaborative results are far from satisfactory. The values reported for reducing sugars are exceedingly variable. In checking over the method for the possible sources of error, the Associate Referee found that differences in the technic of putting the flour in suspension could cause almost as much variation as was shown in the collaborative results. To eliminate this source of error it was found necessary to keep the liquid (while being pipetted into the flask) from coming in contact with the flour. The flour and liquid could then be shaken together to form a suspension instantaneously.

The method as now recommended for further collaborative study is as follows.

REDUCING AND NON-REDUCING SUGARS IN FLOUR

REAGENTS

(a) *Ethyl alcohol.*—95% (by volume).

(b) *Acid buffer solution.*—Make 3 cc. of glacial acetic acid, 4.1 grams of anhydrous Na acetate, and 4.8 cc. of H_2SO_4 (sp. g. 1.84) to 1 liter with water.

(c) *Sodium tungstate.*—12%. Make 12.0 grams of Na tungstate to 100 cc. with water.

(d) *Ferricyanide solution.*—Alkaline 0.10 N. 33.0 grams of pure dry $K_3Fe(CN)_6$ and 44.0 grams anhydrous Na_2CO_3 per liter.

(e) *Thiosulfate solution.*—0.10 N. 24.82 grams of $Na_2S_2O_3 \cdot 5H_2O$ and 3.8 grams of borax per liter.

(f) *Acetic acid-salt mixture.*—200 cc. of glacial acetic acid, 70 grams of KCL, and 40 grams of $ZnSO_4 \cdot 7H_2O$ per liter.

(g) *Combined 2% soluble starch–50% potassium iodide solution.*—Suspend soluble starch in a small quantity of cold water and pour slowly into boiling water with constant stirring. Cool (cooling must be thorough or the resulting mixture will be dark colored), add KI, make to volume, and add one drop of saturated NaOH solution per 100 cc. Use 1 cc.

DETERMINATION

(1) *Maltose.*—Introduce 5 grams of flour into a 100 or 125 cc. Erlenmeyer flask. Tip the flask so that all the flour is at one side and wet the flour with 5 cc. of 95% alcohol. Then tip the flask so that the wet flour is at the upper side and add 43.4 cc. of the acid buffer solution, keeping the solution from coming in contact with the flour until it has all been added to the flask. Then shake the flask to bring the flour into suspension.

Add immediately 2 cc. of the Na tungstate solution and again mix thoroly.

[1] *Cereal Chem.*, 14, 767 (1937).
[11] See footnote on preceding page.

Filter at once (Whatman No. 4 or equivalent), discarding the first 8–10 drops of filtrate. Pipet 5 cc. into a test tube of approximately 75 cc. capacity (Pyrex 1″×8″). Add to the test tube with a pipet exactly 10 cc. of the alkaline Fe(CN)₆ solution, mix, and immerse the test tube in a vigorously boiling water bath. (The surface of the liquid in the test tube should be 3–4 cm. below the surface of the boiling water.)

After the test tube has been in the boiling water bath exactly 20 minutes, cool the tube and contents under running water, and pour at once into a 100 or 125 cc.

TABLE 1.—*0.10 N ferricyanide maltose conversion table*[1]

0.10 N THIOSULFATE	MALTOSE PER 10 G. FLOUR	0.10 N THIOSULFATE	MALTOSE PER 10 G. FLOUR	0.10 N THIOSULFATE	MALTOSE PER 10 G. FLOUR
cc.	mg.	cc.	mg.	cc.	mg.
0.10	618	3.40	373	6.70	166
0.20	608	3.50	367	6.80	161
0.30	598	3.60	360	6.90	156
0.40	588	3.70	353	7.00	151
0.50	578	3.80	347	7.10	145
0.60	568	3.90	341	7.20	140
0.70	558	4.00	334	7.30	135
0.80	550	4.10	328	7.40	130
0.90	542	4.20	322	7.50	126
1.00	534	4.30	315	7.60	121
1.10	527	4.40	308	7.70	116
1.20	519	4.50	302	7.80	111
1.30	512	4.60	295	7.90	106
1.40	505	4.70	288	8.00	101
1.50	499	4.80	282	8.10	96
1.60	492	4.90	276	8.20	90
1.70	485	5.00	270	8.30	85
1.80	478	5.10	264	8.40	80
1.90	472	5 20	257	8.50	76
2.00	465	5.30	251	8.60	71
2.10	458	5.40	244	8.70	65
2.20	451	5.50	237	8.80	60
2.30	445	5.60	231	8.90	56
2.40	438	5.70	225	9.00	51
2.50	431	5.80	218	9.10	46
2.60	425	5.90	213	9.20	41
2.70	418	6.00	207	9.30	36
2.80	412	6.10	201	9.40	31
2.90	406	6.20	195	9.50	25
3.00	398	6.30	188	9.60	20
3.10	392	6.40	182	9.70	15
3.20	385	6.50	176	9.80	10
3.30	379	6.60	171	9.90	5

[1] *Cereal Chem*, 14, 603 (1937).

Erlenmeyer flask. Rinse out the test tube with 25 cc. of the acetic acid-salt solution, add to contents of Erlenmeyer flask, and mix thoroly. Then add 1 cc. of the 2% soluble starch-50% KI solution. Titrate with 0.10 N Na₂S₂O₃ solution to the complete disappearance of the blue color. A 10 cc. micro-buret is recommended for the titration. This titration value represents a definite quantity of reducing sugar per

10 grams of flour, which may be ascertained (as maltose) by consulting the 0.10 N Ferricyanide Maltose Conversion Table (Table 1).

Non-Reducing Sugars.—Pipet 5 cc. of the filtered, clarified flour extract (made according to the above specifications) into an 8″ test tube and immerse in a vigorously boiling water bath. After 15 minutes' boiling, cool the test tube and contents under running water and add 10 cc. of the alkaline 0.10 N Fe(CN)$_6$. Carry out the reduction of the Fe(CN)$_6$ (immersion in boiling water bath for 20 minutes) and the subsequent titration exactly as directed for the determination of maltose (1). Subtract the Fe(CN)$_6$ reduced by the maltose in the flour from the Fe(CN)$_6$ reduced after hydrolysis and obtain the non-reducing sugars represented by the difference (calculated as sucrose) from the Ferricyanide Sucrose Table (Table 2).

TABLE 2.—*0.10 N ferricyanide sucrose conversion table*[1]

0.10 N FERRICYANIDE REDUCED	SUCROSE PER 10 G. FLOUR	0.10 N FERRICYANIDE REDUCED	SUCROSE PER 10 G. FLOUR	0.10 N FERRICYANIDE REDUCED	SUCROSE PER 10 G. FLOUR
cc.	*mg.*	*cc.*	*mg.*	*cc.*	*mg.*
0.10	5	3.00	143	5.90	280
0.20	10	3.10	148	6.00	285
0.30	15	3.20	152	6.10	290
0.40	19	3.30	157	6.20	294
0.50	24	3.40	161	6.30	299
0.60	29	3.50	166	6.40	304
0.70	34	3.60	171	6.50	309
0.80	38	3.70	176	6.60	313
0.90	43	3.80	181	6.70	318
1.00	48	3.90	185	6.80	323
1.10	52	4.00	190	6.90	328
1.20	57	4.10	195	7.00	333
1.30	62	4.20	200	7.10	337
1.40	67	4.30	204	7.20	342
1.50	71	4.40	209	7.30	347
1.60	76	4.50	214	7.40	352
1.70	81	4.60	218	7.50	357
1.80	86	4.70	223	7.60	362
1.90	91	4.80	228	7.70	367
2.00	95	4.90	233	7.80	372
2.10	100	5.00	238	7.90	377
2.20	104	5.10	242	8.00	382
2.30	109	5.20	247	8.10	387
2.40	114	5.30	251	8.20	392
2.50	119	5.40	256	8.30	397
2.60	123	5.50	261	8.40	402
2.70	128	5.60	266	8.50	407
2.80	133	5.70	270	—	—
2.90	138	5.80	275	—	—

[1] Cereal Chem., 14, 767 (1937).

No report on baking test for soft wheat flour was given by the associate referee.

REPORT ON FLOUR-BLEACHING CHEMICALS

By DOROTHY B. SCOTT (U. S. Food and Drug Administration,
New York, N. Y.), *Associate Referee*

Samples for a collaborative study of Munsey's modification of the Kent-Jones and Herd method, *This Journal*, 18, 497, for the determination of small quantities of chlorine in Agene-bleached flour, were sent to nine analysts, in accordance with the recommendation of Subcommittee D.

TABLE 1.—*Chlorine (moisture-free basis)—p.p.m.*

COLLABORATOR	SAMPLE A 5 GRAMS AGENE PER BBL.	SAMPLE B 1 GRAM AGENE PER BBL.	SAMPLE C UNBLEACHED	SAMPLE D BETA-CHLORA BLEACH
R. A. Barackman Victor Chemical Works Chicago, Ill.	7.2	3.1	2.7	154
Robert D'Orazio Schwarz Labs., Inc. New York, N. Y.	6.0	2.3	3.7	83.1
G. M. Johnson Food & Drug Adm. Minneapolis, Minn.	4.0 / 3.7 } 3.9	1.7 / 2.1 } 1.9	0.6 / 1.6 } 1.1	173 / 149 } 161
H. V. Moss Monsanto Chemical Co. St. Louis, Mo.	5.03	1.67 / 1.56 }1.61	1.20 / 1.08 }1.14	127.1 / 119.7 }123.4
V. E. Munsey* Food & Drug Adm. Washington, D. C.	4.5 / 4.3 } 4.4	1.9 / 1.7 } 1.8	1.1 / 0.9 } 1.0	131 / 125 } 128
H. K. Parker* Wallace and Tiernan Co., Inc. Newark, N. J.	4.11 / 3.64 }3.87	1.69 / 1.52 }1.6	0.55 / 0.31 }0.43	112.3 / 123.14 }117.7
T. H. Riggs* Food & Drug Adm. New York, N. Y.	5.5	1.7	1.2	135
Manuel Tubis Food & Drug Adm. Philadelphia, Pa.	6.29	3.02	2.0	102.4*
J. H. Watkins Food & Drug Adm. New Orleans, La.	6.93 / 6.95 }6.94	4.50 / 4.26 }4.38	3.90 / 3.96 }3.93	146 / 144 } 145
Average	5.46	2.38	1.91	127.7

* Aviation gas used for extraction. Parker used high boiling petroleum ether, 60°-100° C.

The method was changed in several minor respects. The analyst was requested to use petroleum ether as a fat solvent because of the difficulty of obtaining lead-free aviation gas, to use 475 cc. of the extract in the determination, to use 475 cc. of the petroleum ether in the blank, and to add 5 cc. more silver nitrate than was specified in the method.

Two samples bleached with known quantities of Agene (nitrogen trichloride) and the unbleached flour were submitted to the analysts. A fourth commercial flour bleached with Beta-chlora was also submitted for a collaborative study of Munsey's method for the determination of large quantities of chlorine in flour, *This Journal,* 18, 500. The results are shown in Table 1.

COMMENTS OF COLLABORATORS

R. A. Barackman.—Please note that the volumetric titration method was used for determining chlorine in Sample D rather than the gravimetric method, which was suggested. Volume of the filtrate: A—277.5 cc. B—437.5 cc. C—437.5 cc. D—100 cc.

G. M. Johnson.—When filtering the petroleum ether extracts, I noticed considerable evaporation of the solvent, particularly because the Büchner funnel I was using (the only one available) did not make a good fit for the filter paper, which made it impossible to get a filtrate free from flour particles regardless of the number of times that it was passed through the paper. I would suggest that aviation gasoline, if obtainable, is superior to petroleum ether. The chloride blank on the reagents was high, amounting to 1.22 cc. of 0.01 $AgNO_3$.

V. E. Munsey.—Your instructions were to use 475 cc. of gasoline extract in this procedure. You will note that my average for 65 samples was only 477 cc. Therefore, you would expect many of the samples to yield less volume than 475 cc. On these particular samples the following results were obtained: 370, 415, 430, 435, 485, 520. With the use of petroleum ether these volumes would be even less. Therefore, in only two cases was it possible to use as much as 475 cc.

H. K. Parker.—Following the method as read, I determined the blank by using 475 cc. of the high boiling petroleum ether (Viking Brand) and evaporating "nearly to dryness" before adding sodium ethylate. A zero blank was obtained. However, when the flour extract was evaporated nearly to dryness, heavy chloride precipitates were observed on Flour B, which was apparently lightly bleached, so the blank determination was repeated except that instead of evaporation "nearly to dryness" the sodium ethylate was added to about the last 7–10 cc. of concentrate. A blank equivalent to 6.75 p.p.m. was obtained. This showed the presence of volatile chlorides, which might have been held back in the fatty extract, depending upon one's judgment as to arrival at the stage of "nearly to dryness," so for the remainder of the determinations the petroleum ether was purified by refluxing over a mixture of sodium-sodium methylate and silver oxide. A blank of 0.3 p.p.m. was obtained when the sodium ethylate was added to the last 10 cc. of the petroleum ether concentrate. I suggest that the blank be determined upon either the gasoline or petroleum ether concentrate or by use of a known chloride-free fat. The latter procedure might be preferable to lessen attack of caustic upon platinum. The Volhard method was used in determination of chlorides in Flour D.

T. H. Riggs.—The Agene method was followed. Aviation gas, lead-free, and petroleum ether, were used, but the petroleum ether results were discarded due to the extremely high blank of the petroleum ether used.

Manuel Tubis.—In the case of Sample D analyzed for Beta-chlora bleach, I used aviation gasoline of doubtful purity and it yielded a high blank. In the Agene method, the values of the filtrates were A—380 cc.; B—430 cc.; C—370 cc. and 475 cc. in the blank. A second set of determinations was made, but owing to the poor conditions and unknown history of the aviation gasoline used the blank was very high and the final results were much lower than those with petroleum ether.

J. H. Watkins.—On account of the volatility of petroleum ether, it seemed advisable to cool the extracts somewhat just before filtering, which was carried out as quickly as possible so as to avoid undue evaporation. Sample D was run according to your directions, but on another sample I avoided filtration and exposure to evaporation of a large surface of flour wet with petroleum ether by withdrawing 100 cc. of extract with a 100 cc. pipet loosely plugged with cotton. The sample had been allowed to stand overnight and the 100 cc. was withdrawn without disturbing the flour layer so that a clear extract was obtained.

DISCUSSION

From the experiences of the analysts, several suggestions for the improvement of the methods should be studied.

Riggs and Watkins have suggested that the flour and gasoline mixture be made up to a definite volume, the flour allowed to settle overnight, and a definite volume syphoned off in the morning without disturbing the flour layer.

This procedure would eliminate the excessive evaporation of the solvent, which is dependent on temperature, rapidity of filtration, and the extent of the surface of the flour exposed in the large Büchner funnel when filtering. There would be no flour particles in the extract and it would also answer the question of the correct quantity of gasoline to be used in the blank. The solvent must be known to be very low in chlorides before it is used.

The collaborators were also asked to determine carotenoid pigments in the flour samples, using Munsey's method, *This Journal*, 21, 339, if a neutral wedge photometer was available, and also the gasoline color values as given in *Methods of Analysis, A.O.A.C.*, 1935, 218. The results are given in Table 2.

TABLE 2.—*Carotenoid pigments as carotene—p.p.m.*

COLLABORATOR	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
G. M. Johnson	0.45 | 0.61 } 0.53	1.23 | 1.45 } 1.34	1.65 | 1.84 } 1.75	0.10 | 0 20 } 0.15
V. E. Munsey	0.8	1.7	2.0	0 3
H. K. Parker	0.924 | 0.825 } 0.874	2.37 | 2.025 } 2.197	2.65 | 2.635 } 2.64	0.250 | 0.225 } 0 237
T. H. Riggs	0.8	1.8	2.2	0.3
M. Tubis	0.53	1.78	2.03	0.40
J. J. Winston	0.81	1.76	2.01	0.27
Nat. Macaroni Mfrs. Assoc. New York, N. Y. J. H. Watkins	0.68 | 0.71 } 0.70	1.40 | 1.49 | 1.52 } 1.47	1.83 | 1.84 } 1.84	0.18 | 0.19 } 0.19
Average	0.72	1.72	2.07	0.27

Parker used the neutral wedge photometer at the New York Station with the 4 inch cell, filter No. 46, and a calibration curve made by J. L. Hogan a year ago.

The agreement of the collaborators' results is very good, and the collaborators report satisfaction with the method.

TABLE 3.—*Gasoline color value*

COLLABORATOR	SAMPLE A	SAMPLE B	SAMPLE C	SAMPLE D
R. A. Barackman	0.77	1.11	1.25	0.60
Robert D'Orazio	0.35	0.97	1.17	0.09
H. V. Moss	0.44 ⎫ 0.445 0.45 ⎭	1.12 ⎫ 1.10 1.08 ⎭	1.42 ⎫ 1.45 1.48 ⎭	0.087 ⎫ 0.096 0.105 ⎭
V. E. Munsey	0.7	1.9	2.2	0.3
H. K. Parker	0.81 ⎫ 0.71 0.63 ⎭	1.47 ⎫ 1.43 1.39 ⎭	1.92 ⎫ 1.87 1.82 ⎭	0.16 ⎫ 0.175 0.19 ⎭
Manuel Tubis	0.33	0.93	1.30	0.08
J. H. Watkins	0.45	1.23	1.66	0.05
Average	0.54	1.25	1.16	0.20

COMMENTS OF COLLABORATORS

R. A. Barackman.—We had considerable difficulty in the use of the Duboscq colorimeter on petroleum ether extracts of the flours so that results were entirely unreliable.

H. V. Moss.—We call your attention to the fact that the gasoline color values were determined on a Duboscq colorimeter.

H. K. Parker.—Solvent was high boiling petroleum ether 60°–100° C. Gasoline color was measured in Hellige-Klett colorimeter at 50 mm. depth. Blue glass light filter. Considerable difficulty was experienced in matching the standard chromate to the higher flour color concentrations due to brown shade. The match is approximated upon intensity rather than shade of color. The most difficult to match seemed to be flour B, which we assume to be the light bleach, and which results are farthest out of line with the Munsey method.

RECOMMENDATIONS

It is recommended[1]—

(1) That the study of methods for the determination of chlorine in flour be continued.

(2) That the method for the measurement of carotenoid pigments in flour given in *This Journal*, 21, 339, be studied next year with the view to substituting water-saturated n-butyl alcohol for the Varsol-alcohol mixture.

(3) That study of the method for the determination of benzoyl peroxide in flour be continued.

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[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).

REPORT ON CARBON DIOXIDE IN SELF-RISING FLOURS

By R. A. BARACKMAN (Victor Chemical Works, Chicago, Ill.),
Associate Referee

Last year, *This Journal*, **21**, 388–403, it was demonstrated that the gasometric method for the determination of carbon dioxide in self-rising flour is subject to errors that result in recovery lower than theoretical. No single factor was found to be responsible. The gasometric procedure was considered to be satisfactory for all practical purposes. Both Whiting, *This Journal*, **15**, 588–591, and Adler, *Ibid.*, **20**, 365–369, suggested that

Carbon dioxide in self-rising flour

DATE OF TESTS (1938)	MIXING DATE 9/2	1 WEEK 9/7	TEST DAYS			AVERAGE CO_2	RECOVERY
			9/12	9/14	9/16		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
L. H. Bailey U. S. Bur. Chemistry and Soils, Washington			—	.682	.672	.677	90.6
R. A. Barackman Victor Chemical Works Chicago	(.692)	(.691)	.656	.677	.675	.669	89.6
Wm. Bruton Kroger Food Foundation Cincinnati			.647	.644	.675	.654	87.6
F. A. Collatz General Mills, Inc. Minneapolis			.660	.655	.663	.659	88.2
J. R. Davies General Foods Corp. Chicago			—	.665	.656	.661	88.4
E. McKim Monsanto Chemical Co. St. Louis			.673	.681	.680	.678	90.7
H. W. Putnam Igleheart Bros. Inc. Evansville			.685	.682	.673	.680	91.0
W. Tholstrup Pillsbury Flour Mills Minneapolis			.676	.672	.685	.678	90.7
L. D. Whiting Ballard and Ballard Co. Louisville			.700	.689	.686	.692	92.6
L. Zeleny U. S. Bur. Agr. Economics Washington			.667	.670	.684	.674	90.2
Average			.670	.671	.675	.672	90.0
Maximum						.692	92.6
Minimum						.654	87.6

a factor be established for the conversion of carbon dioxide recovered to carbon dioxide present. Adler implied that this factor might be dependent on laboratory conditions in various geographical locations. An attempt was made this year to develop such a factor or factors.

A single sample of self-rising flour was prepared having a theoretical carbon dioxide content of .7474 per cent. A soft wheat patent flour having a moisture content of 12.9 per cent was used. The sample was submitted to ten collaborators with the request that determinations of carbon dioxide be made in duplicate on the same calendar days and that modified Method A, *This Journal*, 20, 365–369, be used. Thus the age of the sample and widely varying climatic conditions were eliminated as possible sources of error.

The results from individual collaborators show about the same differences from the average percentage of carbon dioxide recovered as was reported by Adler and by Whiting. There was an indication that a laboratory reporting a result lower than the average with one sample also reports a lower figure with another.

The average percentage recovery of carbon dioxide, 90 per cent of theoretical, is lower than that reported by Adler and by Whiting. Some loss of carbon dioxide occurred between the time of mixing the sample and of testing by collaborators, and because of this no attempt was made this year to establish a factor for converting carbon dioxide found to carbon dioxide present.

It is suggested that next year the Associate Referee submit the ingredients of self-rising flour to collaborators with instructions for mixing immediately before determining the carbon dioxide content. This procedure will eliminate aging effects. The results should show a constant loss of carbon dioxide, characteristic of the gasometric method. A factor may then be established.

There remains to be determined a conversion factor as previously suggested, the influence of flour grade and type, and the percentage recovery of carbon dioxide when variable amounts of sodium bicarbonate (and monocalcium phosphate) are present in self-rising flour.

It is recommended[1] that collaborative testing of the carbon dioxide in self-rising flour be continued along the lines suggested in this report.

REPORT ON MILK SOLIDS IN BREAD

By V. E. Munsey (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The study this year was in accordance with the recommendation of the committee, which included further collaborative work on the citric

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[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).

acid method, the so-called fat method, and the lactose method for the determination of milk solids in bread.

The bread samples studied in previous years by this Referee had been made with known amounts of fresh liquid milk. This year three samples of bread were prepared from known amounts of powdered whole milk. These breads contained 2.6, 5.1, and 7.7 per cent whole milk solids and were numbered, respectively, 1, 2, and 3. They were analyzed for milk solids by four collaborators according to the citric acid procedure on page 224, *Methods of Analysis, A.O.A.C.*, 1935. The results are shown in Table 1.

TABLE 1.—*Milk solids (m.f.b.) by the citric acid procedure (per cent)*

COLLABORATOR	SAMPLE 1		SAMPLE 2		SAMPLE 3	
1	2.9 2.6	Av. 2.8	4.5 5.4	Av. 5.0	10.3	
2	2.6		4.8		5.9	
3	3.2 3.5	Av. 3.4	6.5 6.7	Av. 6.6	9.3 9 1	Av. 9.2
4	3.3 3.5	Av. 3.4	6.4 6.5	Av. 6 5	9.4 9.3	Av. 9.3

The results of Collaborators 3 and 4 are much too high for milk solids on all samples. In view of the satisfactory results from a majority of collaborators in previous years the cause of these high results was investigated. The powdered whole milk used in these breads contained by analysis 1.54 per cent citric acid on the moisture-free basis. This represents a citric acid content on the basis of liquid milk of 0.184 per cent, which is considerably higher than the average value, 0.16 per cent, in liquid milk on which the method of calculation is based. A recalculation of results for Collaborators 3 and 4 based on the actual citric acid content of the milk actually used indicates 2.8 and 3.0 per cent milk solids for Sample 1, 5.5 and 5.5 per cent milk solids for Sample 2, and 8.0 and 7.9 per cent milk solids for Sample 3. Results by Collaborators 1 and 2 would be lowered by about 15 per cent and would be lower than the amount of milk solids present in all except Sample 3, Collaborator 1.

The results in Table 1, together with those of previous years, indicate the variation to be expected among analysts with no significant amount of experience with the methods, and also the possibility that results may not be in close agreement with the actual amount of milk present. The results this year also illustrate the variation based on the natural citric acid content of the milk. It should be emphasized that while the results by this procedure are calculated as whole milk solids, the method actually indicates only the non-fat milk solids unless there is further evidence to establish the corresponding amount of butterfat.

Whole milk solids may also be calculated by determination of the non-fat milk solids by application of the lactose procedure published in *Cereal Chem.* **13**, 541 (1936). The crumbs of the loaves of the same composition as those used for the citric acid procedure were analyzed by three collaborators for milk solids by the same lactose procedure (Table 2).

TABLE 2.—*Milk solids* (*m.f.b.*) *by the lactose procedure* (*per cent*)

COLLABORATOR	SAMPLE 1		SAMPLE 2		SAMPLE 3	
	2.1		5.6		8.3	
1	2.3	Av. 2.4	5.9	Av. 5.6	7.7	Av. 8.0
	2.7		5.2		7.9	
2	3.0		5.3		8.1	
3	2.2	Av. 2.2	5.5	Av. 5.5	8.0	Av. 7.9
	2.2		5.4		7.9	

* Calculated from non-fat milk solids by Referee.

All the results by the lactose procedure are in good agreement with the amount of milk solids actually present. Too much confidence should not be placed in these limited data since a previous study was not so satisfactory and some collaborators have not had success with this long procedure of a biological nature. It may be desirable to include in each batch of samples analyzed by this procedure a sample with a known amount of milk solids to serve as an indication of the proper biological action in the different steps throughout the procedure.

A method based on determination of calcium may be worthy of consideration for judging compliance of the non-fat component of the milk solids in milk bread. The German literature indicates the value of such a method, particularly a paper in *Z. Untersuch. Lebensm.*, **75**, 150 (1938). On the dry basis the results show 40–70 mg. of calcium oxide per 100 grams for water bread and 100–150 mg. per 100 grams for milk bread.

The three samples of bread used in this collaborative study were analyzed by this procedure, which represents a one-third, two-thirds, and all-milk bread with the following results, respectively: 75 mg. per 100 grams, 112 mg. per 100 grams, and 143 mg. per 100 grams. Water breads have analyzed from 28 to 70 mg. per 100 grams, depending on the presence or absence of calcium salts used in so-called dough improvers. The calcium contents of wheat flour and milk are fairly constant, but a variation due to addition of materials containing calcium must be borne in mind in the application of this procedure. It has the advantage of being simple, short, and highly accurate.

The so-called fat method studied is published on page 222, *Methods of Analysis, A.O.A.C.*, 1935. The collaborators were specifically instructed to dry completely the filter pad containing the absorbed fat, to use

(1+4) sulfuric acid instead of (1+1), which is an error in the book, maintain a constant rate of distillation, use carbon dioxide-free distilled water and carbon dioxide-free sodium hydroxide, and to run a blank on reagents to be used as a correction. Since there is some evidence that the sodium hydroxide saponification may not be complete, some of the collaborators were instructed to saponify with the 4 cc. of soda-glycerol mixture under the official Reichert-Meissl method instead of with 1 cc. sodium hydroxide (1+1) specified in the method. The breads submitted were of the same composition and identity as those analyzed in Tables 1 and 2.

TABLE 3.—*Results on % total fat and milk solids (m.f.b.) by so-called fat method*

COLLABORATOR		SAMPLE 1		SAMPLE 2		SAMPLE 3	
				per cent			
1	Fat	5.1 / 5.0	Av. 5.1	5.7 / 5.7	Av. 5.7	6.4 / 6.4	Av. 6.4
	Milk Solids	2.2 / 2.1	Av. 2.2	4.4 / 5.5	Av. 5.0	6.5 / 4.6	Av. 5.6
2	Fat	5.0-5.0, Av. 5.0		5.7-5.7, Av. 5.7		6.2-6.3, Av. 6.3	
	Milk Solids	2.5-2.7, Av. 2.6		5.0-4.6, Av. 4.8		7.0-6.8, Av. 6.9	
3	Fat	—		5.7-5.0, Av. 5.4		5.6-5.8, Av. 5.7	
	Milk Solids	—		6.4-6.9, Av. 6.7		9.3-9.2, Av. 9.3	
4	Fat	4.8-4.9, Av. 4.9		5.6-5.6, Av. 5.6		6.3-6.3, Av. 6.3	
	Milk Solids	2.9-2.9, Av. 2.9		5.2-5.3, Av. 5.3		7.8-7.9, Av. 7.9	
5	Fat	5.1-4.9, Av. 5.0		5.7-5.7, Av. 5.7		6.3-6.3, Av. 6.3	
	Milk Solids	2.3-2.3, Av. 2.3		4.0-4.2, Av. 4.1		7.0-6.5, Av. 6.8	
6	Fat	4.9-4.9, Av. 4.9		5.6-5.7, Av. 5.7		6.2-6.2, Av. 6.2	
	Milk Solids	2.0-2.2, Av. 2.1		3.9-4.3-3.7-3.6 Av. 3.9		6.7-6.0-6.9-8.0 Av. 6.9	

These results (Table 3) show good agreement on total fat and confirm previous studies. The results for milk solids on Sample 1, and some of the results on Samples 2 and 3 are good. In general, the results indicate variations from the actual amount of milk solids present similar to those reported in previous studies. The results of Collaborators 2 and 3 by saponification of the fat by the glycerol-soda procedure indicate no ma-

terial improvement. However, as the result of recent work by the Associate Referee it is recommended that the glycerol-soda saponification be used in place of the sodium hydroxide on future analysis with this method.

Results by the so-called fat method are necessary to establish the butterfat equivalent to the non-fat component. While the results are not in all cases close to the actual amount of milk solids present, they do indicate during the past three years' study the probable variation in results expected and a fair estimation of the amount of butterfat present.

By using the average of three closely agreeing so-called fat numbers a closer estimation of milk solids based on butterfat present may be expected.

The Associate Referee expresses appreciation of the cooperation of the following collaborators who took part in this study: A. H. Wells, Los Angeles Station, Food and Drug Administration; C. E. Hyndsy, Department of Agriculture and Markets. Albany, New York; L. W. Ferris, Buffalo Station, Food and Drug Administration; J. H. Watkins, New Orleans Station, Food and Drug Administration; R. Carson, Department of Agriculture, Ottawa, Canada; D. A. Magraw, American Dry Milk Institute, Inc., Chicago, Ill.; Charles Hoffman, Ward Baking Company, New York City; George H. Marsh, Division of Agricultural Chemistry, Montgomery, Ala.

RECOMMENDATIONS[1]

It is recommended—

(1) That the citric acid procedure for calculating milk solids be not further studied collaboratively for the present.

(2) That the lactose procedure be more extensively studied collaboratively.

(3) That the so-called fat method be not further studied collaboratively in the present form.

(4) That study be made of a method based on the direct saponification and distillation of the bread samples without extraction of the fat for estimation of butterfat content.

REPORT ON COLD WATER EXTRACT IN FLOUR

By H. C. FELLOWS (Agricultural Marketing Service,
Washington, D. C.), *Associate Referee*

Collaborative studies on cold water extract in flour were continued this year, and special attention was given to the following points:

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(1) Grade of filter paper—XX 588 folded filter paper, 18½ cm. diameter, Carl Schleicher & Schüll.

(2) Time of extraction—40 minutes.

(3) Extraction temperature—ice bath (32° F.).

(4) Drying temperature—Approximately 100° C. in vacuum oven to constant weight.

A soft wheat "patent" flour, a soft wheat "straight grade" flour, and a soft wheat "first clear" flour were used in these studies. The collaborative results are given in the table.

Cold water extract, per cent

COLLABORATOR	PATENT FLOUR	STRAIGHT FLOUR	FIRST CLEAR FLOUR
1	4.14	4.06	4.07
2	4.51	4.48	4.63
3	4.22	4.13	4.17
4	4.47	5.04	4.47
5	4.15	4.31	4.37
6	4.17	4.13	4.09
7	3.98	3.98	3.87
8*	4.20	4.02	4.01
8A†	4.17	4.13	4 03

* Filtered as recommended in the method.
† Centrifuged for 10 minutes at high speed and then filtered.

The results obtained by the various collaborators are on the whole in close agreement. The centrifuging process does not have any material effect on the final results but it does speed up filtration.

One collaborator reported his results as dried at 100° C. in the air oven, and the data obtained by him are in line with the findings of the other collaborators, who used the vacuum oven at approximately 100° C.

The collaborators were the following:

1. F. A. Collatz, General Mills, Inc., Minneapolis, Minn.
2. M. H. Neustadt, Agricultural Marketing Service, Washington, D. C.
3. L. W. Haas, The W. E. Long Company, Chicago, Ill.
4. Rae H. Harris, Agr. Expt. Station, Fargo, N. D.
5. M. J. Blish, Agr. Expt. Station, Lincoln, Nebr.
6. Howard M. Simmons, Mid-West Laboratories Co., Inc., Columbus, Ohio.
7. C. G. Harrel, Pillsbury Flour Mills Co., Minneapolis, Minn.
8. H. C. Fellows.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method be rewritten [revised method was published in *This Journal*, 22, 76 (1939)].

(2) That the method then be adopted as official (first action).

(3) That further studies be carried on with other cereals.

No report on ergot in flour was given by the associate referee.

REPORT ON PROTEOLYTIC ENZYMES

By Quick Landis (The Fleischmann Laboratories,
New York City), *Associate Referee*

Both chemical and physical methods have been devised for the measurement of proteolytic activity. Generally speaking, the former determine directly an increase in split peptid linkages, and the latter a change in the colloidal properties of the substrate, which may or may not be a direct consequence of peptid link hydrolysis. Marked changes in physical properties frequently appear long before chemical methods can detect any appreciable change.

The numerous ways in which complex protein molecules can be decomposed is in full harmony with the experimental recognition of various types of specific proteolytic enzymes.[1] Thus, although the methods proposed for study may give only a measure of over-all conversion one should not fail to remember that concurrent specific processes, including "activation," contribute to the final observation. A single method can seldom if ever give a complete picture of the activity of any food sample.

Methods may be further classified as autolytic or analytic. Each class has its own function in enlarging knowledge of the product under consideration. In some cases, of which flour is representative, the results of an autolytic method are perhaps more susceptible of translation to certain practical applications, but for concentrates autolytic methods are usually of little value.

It is therefore proposed to submit three separate classes of methods to simultaneous investigation. The extremely low activity exhibited by flours is a great handicap, but if improvements can be devised a limited collaborative effort may be made. Among the chemical methods developed, the Willstätter alcoholic titration of soluble free carboxyl groups is probably most sensitive and it is proposed to continue the investigation of Balls and Hale, *This Journal*, 18, 135–40, on this method. Probably the most promising autolytic physical method for flour, involving measurement of the softening of a flour paste, was also reported by Balls and Hale, *Ibid.*, 19, 372–3.

If the conditions can be adequately standardized this method may be of practical application. The Associate Referee has also been engaged in the development of a highly sensitive analytic physical method, utilizing

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[1] Bergmann and Ross, *J. Am. Chem. Soc.*, 58, 1503 (1936).

the *change* produced in gelation capacity of a gelatin substrate by proteolytic enzymes.[1] As detailed at present the method is somewhat cumbersome, although readily detecting differences among samples with activities still less than those exhibited by flour. It is hoped that simplification can be achieved without loss of sensitivity.

It is recommended[2] that these three types of methods be investigated.

REPORT ON COLOR IN FLOUR

By H. K. PARKER (Wallace & Tiernan Co., Newark, N. J.), *Associate Referee*

In a short review of the previous collaborative work to develop a method and a machine to measure the color of flour and bread by reflectance, perhaps it should be pointed out that a colorimeter[3] has been developed whereby the color of the sample can be matched by means of color cards mounted upon a rapidly rotating disk (Maxwell Disk). Nine machines were lent to different laboratories where flour and bread were examined and judged almost daily. It is significant to note that with one exception neither the machine nor the method proved satisfactory enough for the laboratory to wish to purchase the machine. The one purchaser apparently finds the machine more useful for colored materials other than flour or bread.

The difficulties encountered seem to be with the preparation of the sample and more particularly with the variation in eye reaction of the various observers. Besides these objections most operators complain that the measurement is time consuming and causes fatigue due to eye strain. Apparently quite concordant results can be obtained by the same observer upon the same sample of flour, and in practice it was expected that one observer could be used in each laboratory to establish a standard flour that could be prepared each week and used to Pekarize against the various unknowns. This procedure has not worked out too well.

Moreover, in practice two different firms with laboratories situated at different cities attempted to check the same sample on various occasions and were unable to do so satisfactorily. This work was done in addition to the A.O.A.C. collaborative tests, *This Journal*, 18, 593; 19, 569. In speculation that the preparation of the sample had much to do with discrepancies, since it has been shown that the rate of drying affects the color and quite likely a difference in humidities at different cities occurs, an attempt was made to obviate this difficulty as proposed in the oral report given at the 1937 A.O.A.C. meeting, *Ibid.*, 20, 382. Upon the

[1] Landis and Frey, *Cereal Chem.*, 15, 91–101 (1938).
[2] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).
[3] *Cereal Chem.*, 10, 437 (1933).

suggestion of Miss Nickerson, Messrs. Rouse and Shearer of Trenton, N. J., who have prepared sub-standards for the silk industry, were contacted. Enamelled steel slabs were prepared by them to match wet flour color. Before sending these slabs out to various collaborators it was found in this laboratory that not much better checks could be obtained on the same slab by different observers than previously and for this reason the substandard was not sent out. Hence it appears that the various observers' eyes differ so greatly in this sort of comparison work that further effort with the N-A colorimeter for flour and bread color measurement has been suspended and the collaborative machines have been called in.

The measurement of flour color by reflectance has much merit, since it closely follows what the miller or baker sees on the Pekar test or finished bread. The problem of evaluation of the various factors is somewhat difficult because of the ever-changing values during aging and drying. It is probable that samples held at the same moisture content (assuming no color change due to enzymatic action) could be measured reproducibly.

Some work was done to study the use of the photoelectric cell upon dry flours but to date differences found have been too small to record accurately, for while the brightness as a whole may be measurable, there seems to be no simple means of evaluating the various shades of color other than by the use of light filters.

RECOMMENDATIONS[1]

It is recommended—

(1) That further collaborative work on measurement of flour and bread color by the N-A colorimeter be dropped.

(2) That study be continued on the sampling and preparation of flour and bread for color measurements by the photoelectric cell method based on reflectance.

REPORT ON SOYBEAN FLOUR IN FOODS

By J. W. HAYWARD (Archer-Daniels-Midland Co., Minneapolis, Minn.), *Associate Referee*

Nitrogen-Free Extract Method.—In this laboratory several successful attempts have been made to determine the quantity of soybean flour in sausage by a nitrogen-free extract method, which consists of analyzing the sausage for moisture, protein, fat, fiber, and ash; calculating the nitrogen-free extract by difference; and multiplying the result by a factor to give the soybean flour content of the sausage.

The underlying principle necessary to the success of this proposed procedure is based on the assumption that there is little or no nitrogen-free

[1] For report of Subcommittee D and action by the Association, see *This Journal, 22,* 68 (1939).

extract present in meat (liver products being the exception), while the nitrogen-free extract of soybean flour is quite constant, running about 30 per cent.

NITROGEN-FREE EXTRACT METHOD

(All references given are to *Methods of Analysis, A.O.A.C.*, 1935.)
Amount of sample...........................500 grams preferred
Preparation of sample.....................Chap. XXVIII, sec. 1(c), p. 353.
Analysis of ground fresh sample:
    Moisture..............................Chap. XXVII, sec. 2, p. 353.
    Protein...............................Chap. XXVIII, sec. 3(b), p. 353.
    Fat...................................Chap. XXVIII, sec. 6, p. 354.
        Regrind for complete extraction.
Analysis of dried extracted sample, Chap. XXVIII, sec. 1(c), p. 353:
    Moisture..............................Chap. XXVII, sec. 2' p. 335.
    Protein...............................Chap. II, sec. 19, p. 23.
    Fat...................................Chap. XXVIII, sec. 6, p. 354.
    Fiber (crude). ....Chap. XXVII, sec. 25, p. 340.
    Ash............... ....Chap. XXVII, sec. 8, p. 336.

Calculate the fiber and ash found in dried extracted material to the moisture and fat basis of the freshly ground sausage, using the following formula:

$$\frac{100 - (\% \text{ moisture in meat} + \% \text{ fat in meat})}{100 - (\% \text{ moisture in extract} + \% \text{ fat in extract})} \times \% \text{ fiber in extract or } \% \text{ ash in}$$

extract = per cent fiber or ash in original material.

Calculate nitrogen-free extract, using actual and calculated values for freshly ground sample.

Express the nitrogen-free extract in terms of soybean flour by using the factor 100/30 as follows:

Nitrogen-free extract × 100/30 = amount of soybean flour added to sausage.

It is not necessary to make a protein determination on the extracted residue except to have a check on the analysis of the original meat. The calculated result should check the analysis of the original material by 0.2 per cent.

Immunological Method by John H. Glynn, M. D., of Armour Laboratories, Chicago, Ill.—The following account is taken from a preliminary report submitted by the Armour laboratories.

We have recently obtained accurate quantitative results by the use of an immunological method that is both simple and rapid. The method is based on a quantitative precipitin test, the "optimal proportions" reaction, first described by Dean and Webb[1] and subsequently proved by Taylor, Adair, and Adair[2] to be well within the limits of accuracy of the best known chemical methods.

The test depends on the fact that in any antigen-antibody titration system the velocity of the reaction is related to the proportion of antigen to antibody. Thus, for a given antibody, precipitation is most rapid when the ratio of antigen to antibody is at an optimum that can be readily

[1] *J. Path. Bact.*, **29**, 473 (1926).
[2] *J. Hyg.*, **32**, 340 (1932).

determined. This optimum ratio is a constant for each antibody solution and is independent of the concentrations of either antigen or antibody in any specific test.

For example, suppose a given antigen reacts most rapidly with a given antibody at a ratio of 1 to 50; that is, one part of antigen forms a precipitate with 50 parts of antibody at a faster rate than with 45 or 55 parts of antibody. In fact, any ratio other than 1 to 50 will be slower than this optimum. Then, since this optimum ratio is a constant, the actual concentration of reagents may vary within fairly wide limits. It may be 3 to 150, or 10 to 500, or 25 to 1250.

It is a simple matter to standardize any particular antibody against a known antigen in terms of optimal ratio. If this ratio is used the concentration of antigen in any unknown mixture can be determined.

In the specific instance of quantitative assay of soybean protein in sausage, the test is performed as follows:

Rabbits are immunized against a 5 per cent sodium chloride extract of soybean flour. For practical purposes, it is unnecessary to use purified glycinin. Several courses of injections over three or four months are usually necessary to produce a serum of satisfactory potency. The serum is collected and standardized against known soybean flour extract. Its optimal ratio is determined as accurately as possible. This ratio is then a constant for that particular serum.

Sausage containing soybean flour is extracted with 5 per cent sodium chloride. This unknown extract is then titrated against the standard serum and its ratio determined. By dividing the test ratio by the standard ratio the percentage of soybean protein in sausage is given.

For example, a standardized serum had a ratio of 1:30 against pure soybean flour extract. An extract of sausage gave a ratio of 1:3 with this serum. Therefore the sausage contained 10 per cent of soybean flour. The accuracy of the method is limited only by the care with which the test is performed; that is, the ability of the operator to distinguish the most rapidly precipitating tube in a rack of ten or twelve tubes. The specificity of the method is limited only by the phylogenetic relationship of the protein mixture under test, a well established immunological fact.

Details of the test will appear in a subsequent publication.

RECOMMENDATIONS[1]

It is recommended that work be continued on the determination of soybean flour in sausage and other foods following the proposed nitrogen-free extract and immunological methods.

No report on macaroni was given by the associate referee.

REPORT ON WHOLE WHEAT FLOUR

By C. S. LADD (State Food Commissioner and Chemist, Bismarck, N. D.), *Associate Referee*

The determination of the whole wheat content of baked products is regularly made by calculation from the crude fiber content of the product. Such calculation is theoretically sound, but in the experience of the Associate Referee the results ordinarily obtained often show percentages not compatible with the actual formulas used in the manufacture of the products. It appears that the difficulties inherent in the crude fiber determination as applied to commercial feeding stuffs are multiplied, or at least are of more significance, in dealing with a product such as whole wheat flour.

The official crude fiber determination has always been open to several serious objections when applied to the determination of the actual fiber content of plant materials, *This Journal*, **2**, 32. Recent investigations indicate that although reproducible results on the same sort of materials are obtained with the standardized technic, they have but little significance when applied to different sorts of plant materials.[1,2] This is explained by the fact that this determination accounts for a variable proportion of the cellulose and lignin present, depending somewhat upon their mutual interference in the digestion.

Another factor influencing the fiber determination that is of recognized importance is the degree of fineness of the samples, *This Journal*, **5**, 56. According to Korfhage[3] fineness of more than 60 mesh gives inconsistent results. This may be of little importance in the analysis of feeding stuffs where the particle size can be selected in grinding the sample, but in the case of flours the results are open to this objection, and it is evident that a proportion of the sample is lost through the linen filter. Pickel[4] found that in the case of cacao products the loss is as high as 0.46 per cent of solids. Losses due to colloidal suspensions in the sodium hydroxide digesting solution have not been measured, and perhaps are insignificant. Various attempts to avoid loss of sample through the filter, such as the modification of Sweeny,[5] et al.,[6,7] (use of a 3.5 per cent sodium hydroxide solution for the caustic digestion and omission of the filtration at the midpoint) were moderately successful. However, this method consistently gives high results compared with those obtained by the regular A.O.A.C. procedure, *This Journal*, **2**, 132; **3**, 256; **4**, 39, with one exception[8] and for this reason has been discounted.

[1] *Science Progress*, **30**, 702 (1936).
[2] *J. Agri. Science*, **25**, 529–40 (1935).
[3] Minn. State Dairy and Food Com., Div., Feed Insp. Ann. Rpt., **4**, 32–8 (1922).
[4] *J. Ind. Eng. Chem.*, **2**, 280 (1910).
[5] U. S. Dept. Agr. Bur. Chem. Bull., **137**, p. 157.
[6] *J. Ind. Eng. Chem.*, **4**, 600 (1912).
[7] Landw. Jahrb. Schweiz., **48**, 909 (1934).
[8] *J. Am. Assoc. Cereal Chem.*, **7**, 208 (1922).

Although the regular official procedure is recommended by the Association for the investigation of bread and baked products, *This Journal*, **16**, 518; **17**, 404, in cases where the percentage of whole wheat flour in a product is calculated from the crude fiber content a slight variation in the result may give rise to a large error in the estimation of whole wheat content.

To obviate the difficulties inherent in the present method some of the newer methods proposed for actual cellulose determination were investigated.

The method of Kurschner and Hanak[9] was selected for the first experimentation.

EXPERIMENTAL

Samples were obtained of two grades of whole wheat flour, designated here as "Whole Wheat Medium Grind" and "Whole Wheat Fine Grind." Bread containing some whole wheat flour of known composition was also obtained, as well as samples of the flours used in its manufacture. For purposes of comparison regular analyses by the official methods were made first. The results, expressed as per cent, follow:

	Moisture	Protein	Fat	Crude Fiber	Ash
1. Whole Wheat Flour Medium	11.92	16.68	1.87	2.38	1.65
2. Whole Wheat Flour Fine	12.52	16.78	1.93	2.29	1.60
3. Bread (⅓ whole wheat)	36.57	10.84	1.37	0.84	2.06
4. Whole Wheat Flour	9.94	17.78	1.85	2.48	1.61
5. Straight Flour	11.62	14.66	0.96	0.31	0.49

Calculated to a dry basis the crude fibers found above are: (1) 2.70, (2) 2.62, (3) 1.32, (4) 2.75, and (5) 0.35 per cent.

When the Sweeney modification of the crude fiber method was used, the results obtained compared with those obtained by the official method as follows:

			per cent		
1. Whole Wheat Flour Medium	2.40	2.35			Av. 2.38[1]
	2.49	3.27	2.83	2.84	
	3.06	2.90	2.93	3.32	
	3.42	2.84	2.70	3.69	Av. 3.02[2]
2. Whole Wheat Flour Fine	2.32	2.25			Av. 2.29
	2.51	3.00	2.87	2.60	
	2.64	2.55	2.62	2.54	
	2.73	3.02	2.83	2.76	Av. 2.72

[1] A.O.A.C. method.
[2] Sweeney modification and moisture and fat extracted by official short method.

These results support the findings of the investigators mentioned previously in that they average considerably higher than do those by the official method and also lack consistency.

[9] *Z. Untersuch. Lebensm.*, **59**, 484 (1930).

The same samples were used with the Kurschner-Hanak[9] method, which follows:

The defatted sample is intimately mixed with 60 cc. of 80% AcOH and 1.5 cc. of HNO_3 (d. 1.4) in a flask fitted with a ground-in air condenser. The mixture is gently refluxed for 25 minutes and then filtered through a tared Gooch or alundum crucible previously wetted with the acid mixture. The residue is washed successively with 7–10 cc. of the acid mixture, hot water, alcohol, ether, 1–2 cc. of the acid mixture, and finally with hot water sufficient to remove all traces of the acid, the flask being rinsed with each washing. The residue is dried at 105° and weighed.

According to the authors this method gives most consistent results and extracts pure cellulose.

The following results were obtained:

	Cellulose (*per cent*)				
1. Whole Wheat Flour Medium					
Moisture and fat extracted by short official method	2.08	2.34	2.20	2.38	Av. 2.25
Fat not extracted	2.19	2.21	2.23	Av. 2.21	
2. Whole Wheat Flour Fine					
Moisture and fat extracted by short official method	1.81	2.21	2.10	Av. 2.04	
Fat not extracted	2.16	2.28		Av. 2.22	
3. Bread ($\frac{1}{2}$ whole wheat, moisture reduced to 6.62%)					
Moisture and fat extracted by short official method	1.64	1.55	1.52		
	1.46	1.49	1.55		
	1.55			Av. 1.54	
	dry basis			Av. 1.65	
Moisture and fat extracted by regular A.O.A.C. method	1.29	1.22	1.85		
	1.55	1.81	1.71		
	1.81	1.68	2.06		
	2.15	2.15	1.65		
	1.50	1.74	1.50		
	1.88	1.65		Av. 1.75	
	dry basis			Av. 1.87	
4. Whole Wheat Flour					
Moisture and fat extracted by short official method	2.14	2.45	2.13		
	2.15			Av. 2.13	
	dry basis			Av. 2.37	
Moisture and fat extracted by regular A.O.A.C. method	2.28	2.25	2.56		
	2.56	2.65	2.27		
	2.52	2.43	2.67		
	2.45	2.53	2.77		
	2.88	2.67	2.71	Av. 2.59	
	dry basis			Av. 2.88	
5. Straight Flour					
Moisture and fat extracted by short official method	0.32	0.54	0.30	Av. 0.39	
	dry basis			Av. 0.44	
Moisture and fat extracted by regular A.O.A.C. method	0.24	0.34	0.47		
	0.50	0.65	0.49		
	0.16	0.23	0.55		
	0.70	0.92	0.73	Av. 0.58	
	dry basis			Av. 0.66	

When the determinations were made without extraction of moisture and fat the following results were obtained:

			per cent		
Sample 3 (Bread)	2.15	2.06	Av. 2.11	dry basis	2.26
Sample 4 (Whole Wheat Flour)	2.24	2.84	Av. 2.59	dry basis	2.88
Sample 5 (Straight Flour)	0.85	0.83	Av. 0.84	dry basis	0.95

It was naturally expected that the samples that had not been defatted would not give reasonable results.

It is apparent that too wide a spread obtains between determinations. However, all results are reported, and undoubtedly lack of experience with the technic of the determination was responsible for some of the differences. It also seems possible that some high results may be due to insufficient washing of the residue in the alundum crucible or to the fact that that part of the material soluble in the acid mixture was being precipitated in the pores of the crucible. To avoid such difficulty the cellulose was filtered on an asbestos pad in a Gooch crucible. The results follow:

			per cent		
Sample 3 (Bread)	0.96	1.09	Av. 1.02	dry basis	Av. 1.09
	0.96	1.13			
Sample 4 (Whole Wheat Flour)	2.12	2.05	Av. 2.07	dry basis	Av. 2.30
	2.05	2.07			
Sample 5 (Straight Flour)	0.09	0.12	Av. 0.10	dry basis	Av. 0.11
	0.10	0.10			

Mixtures of the two flours gave the following results:

	per cent			*per cent*	
¼ Whole Wheat Flour	0.61	0.62	¾ Whole Wheat Flour	1.63	1.64
¾ Straight Flour	0.61	0.61	¼ Straight Flour	1.64	
	Av.	0.61		Av.	1.64
	dry basis	0.69		dry basis	1.83

These percentages are in agreement with values calculated from the percentages immediately preceding. The agreement between results also seems satisfactory although the total number of analyses made by the Kurschner-Hanak method is not large enough to justify definite conclusions.

CONCLUSION

The results obtained indicate that it may be possible to substitute a cellulose determination for the official crude fiber method in the investigation of the whole wheat content of whole wheat products and thereby save considerable time. Determinations made with the Gooch filter gave more consistent results than those made with an alundum crucible. Further work will be necessary to establish reliability of the method although it does show promise at present.

It is recommended[1] that the Associate Referee continue his studies on

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[1] For report of Subcommittee D and action by the Association, see *This Journal*, **22**, 68 (1939).

methods of determining cellulose as an index of the whole wheat flour content of cereal foods.

ACKNOWLEDGMENT

Acknowledgment is made by the Associate Referee of the analytical work contained in this report by E. J. Hennessy and O. Maercklein, Assistant Chemists in the Associate Referee's laboratories.

REPORT ON PHOSPHATED FLOUR

By J. R. DAVIES (Calumet Baking Powder Division, General Foods Corp.), *Associate Referee*

The results of the work on phosphated flours carried on during 1938 confirm those of Gustafson,[1] Shuey,[2] and Barackman and Vaupel.[3] The method of Gustafson for the determination of the ash of the original flour is based upon removal of added inorganic ingredients by digestion in and sedimentation from carbon tetrachloride.

The Associate Referee found the Gustafson method to be excellent for the removal of added salts (monocalcium phosphate, sodium bicarbonate, and sodium chloride) from flours, except in the case of self-rising flours that had been exposed to humid conditions. In this case sufficient moisture is absorbed to cause formation of "shot-falls" of high sodium chloride content. These balls collect in the flour mass on the surface of the carbon tetrachloride and give high ash values. The difference between fresh and aged self-rising flours is shown in the percentage of ash due to the sodium chloride content. Analyses for the sodium chloride content of the fresh and aged self-rising flours show differences in the same range as found in the ashes from the same. Grinding the flour to pass several different sized screens before extraction with carbon tetrachloride does not eliminate the salt effect. In fact, grinding the flour in a mortar and pestle gives slightly higher values; namely, 0.732 per cent ash in the unground, and 0.839 per cent ash in the ground extracted flour. Analyses of these ashes for sodium chloride content show substantially this difference.

The ashes of flour extracted with carbon tetrachloride are generally slightly lower than those of the control flour. This error was reported by Gustafson,[1] Shuey,[2] and Barackman and Vaupel.[3] The Associate Referee's results show 0.358 per cent ash on the control flour and 0.342 per cent ash on the extracted control flour.

Results of experiments indicate that two extractions of the phosphated and the self-rising flours with 50 cc. portions of carbon tetrachloride in a

[1] *Cereal Chem.*, **8**, 475–81 (1931).
[2] *Ibid.*, **12**, 289–93 (1935).
[3] *Ibid.*, 486–93.

250 cc. Squibb separator give separations of the added salts that are in close agreement with results obtained by centrifuging as in the Gustafson method. A comparison of the results of analyses for ash after extraction by (a) centrifuging, and (b) separator are shown as follows:

Ash of original flour after extraction by carbon tetrachloride—Reported on 15% moisture basis

	Collaborator A Centrifuge *per cent*	Associate Referee Sedimentation *per cent*
Plain flour	0.343	0.343
Phosphated flour	0.356	0.353
Self-rising flour	0.354	0.351
Phosphated flour—aged	0.353	0.357
Self-rising flour—aged	0.724	0.732
Control flour (not extracted)	0.364	0.361

It is recommended[1] that further study be made of methods for determining the ash of the original flour in phosphated and self-rising flours, and especially of old self-rising flours, with special attention given to (a) analyses for sodium chloride content of self-rising flours and the ash of the extracted flours; and (b) the means other than extraction with carbon tetrachloride for 100 per cent separation of the sodium chloride from old self-rising flour.

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[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 68 (1939).

TUESDAY—AFTERNOON SESSION

REPORT ON STANDARD SOLUTIONS

By R. L. Vandaveer (U. S. Food and Drug Administration,
New Orleans, La.), *Referee*

There are two methods by which the chemist determines the effective strength of a standard solution: (1) A substance of known strength and purity can be diluted to a definite volume; (2) the solution may also be titrated against a definite weight of a pure chemical compound (usually called a primary standard). To eliminate the possibility of dilution error, the determination of normality after preparation of the solution is preferable. Obviously, the standardization of a solution's concentration by both of the above general methods or by more than one primary standard substance should yield a result that is closer to the solution's absolute strength than if only a single method of determining the normality is used.

It is logical that in determining the strength of any one solution, the method chosen should only measure (1) the molecule or (2) the active part of the molecule which is involved in the chemical reaction during the determination. For example: In the standardization of sulfuric acid solution, aside from the analytical difficulties involved, many chemists prefer to measure the effective acid strength rather than determine the relatively inert SO_4 radical.

Thus it is believed desirable—whenever there is available more than one equally accurate direct method for determining the strength of the standard solution under study—not to give preference to one procedure, but to submit at least two to this Association for consideration.

RÉSUMÉ OF COLLABORATIVE EFFORT

The reported studies on standardization of acid solutions this year deal principally with methods for standardization of hydrochloric acid solutions by precipitation with silver, and by direct titration with both recrystallized borax and sodium carbonate as primary standards. Collaborative results reported indicate that the borax and sodium carbonate methods have considerable merit. Normalities obtained by the silver chloride method were disappointing, but rather expected, since no provision is made in this procedure for solubility.

Studies were made on iodine and thiosulfate solutions for the first time by an associate referee. By the two methods proposed for standardization of iodine solution, the associate referee obtained results that agree to within 1 part in 2000. The associate referee suggests further study on methods for thiosulfate, which suggestion is approved by the Referee.

Last year no recommendation was made for any official action on the tentative indirect method for standardization of hydrochloric acid solution, *Methods of Analysis, A.O.A.C.*, 1935, 682, since it was considered to be desirable to have a direct method available before this recommendation was submitted. The collaborative results on this hydrochloric acid solution were obtained by titration against sodium hydroxide solution. At the same time, the collaborators standardized the sodium hydroxide solution against pure acid potassium phthalate. The data obtained are reported in *This Journal*, 21, 411. These results on both methods are in such good agreement as to warrant both being recommended as official.

In addition to the methods that have been studied, there is throughout the present book of methods a host of other standard solutions in general use. All these methods and many others, should be considered by the Referee on Standard Solutions. Naturally, those of most importance deserve early consideration. Next year, in addition to acidimetry and iodometry, the Referee proposes to have studies conducted in the field of argentometry and permanganometry.

RECOMMENDATIONS[1]

It is recommended—

(1) That the methods for the preparation and standardization of solutions of sodium hydroxide, *Methods of Analysis, A.O.A.C.*, 1935, 681, be adopted as official (first action).

(2) That the method for the preparation and standardization of hydrochloric acid solutions, *Ibid.*, 682, be adopted as official (first action).

(3) That the method submitted by the Referee for the standardization of acid solutions with sodium carbonate be adopted as tentative.

(4) That the method submitted by the Referee for the standardization of acid solutions with borax be adopted as tentative.

(5) That studies on the standardization of hydrochloric acid solutions by the silver chloride procedure, *Ibid.*, 23, be discontinued.

(6) That the preparation and standardization of sulfuric acid solutions be studied.

(7) That the methods submitted by the associate referee for the standardization of iodine solutions be made tentative.

(8) That studies on methods for the standardization of thiosulfate be continued.

(9) That the preparation and standardization of potassium permanganate solutions be studied.

(10) That the preparation and standardization of silver nitrate and thiocyanate solutions be studied.

REPORT ON STANDARDIZATION OF ACIDIMETRIC SOLUTIONS

By R. L. VANDAVEER (U. S. Food and Drug Administration, New Orleans, La.), *Referee*

Of all the well-known procedures for standardizing acid solutions reported in the literature, the determination of the acid titer of a solution by means of borax $(Na_2B_4O_7.10H_2O)$[1] and of sodium carbonate (Na_2CO_3)[2] as primary standards appears to be foremost. These substances can be prepared pure, and they qualify in other respects as primary standard substances for acidimetry. If an accurate barometer is available, an accurate standard solution of hydrochloric acid may be prepared from the well-known constant boiling acid procedure. The determination of normality by precipitation and weighing as silver chloride also deserves consideration. Consequently, collaborative work (constant boiling HCl excepted), was limited to the above-mentioned methods.

To each collaborator was submitted a portion of a standard solution of hydrochloric acid prepared by the Referee from constant boiling HCl to be exactly 0.1 N at 20° C. The procedure recommended by Foulk and Hollingsworth[3] in obtaining constant boiling acid was followed. This 0.1 N factor on the collaborative solution was also checked through standard sodium hydroxide against acid potassium phthalate, *Methods of Analysis*, *A.O.A.C.*, 1935, 681, independently, by S. Alfend and the Referee.

In Table 1 are reported the collaborative efforts on this solution; each normality value was corrected to 20° C.

COMMENTS BY COLLABORATORS

G. M. Johnson.—There is considerable variation between the normality as obtained by precipitation as silver chloride and by direct titration against sodium carbonate or borax. This may be due to improper condition of precipitation and disregard for the solubility of the silver chloride. The standardization with borax was the most satisfactory. A large weight and a sharp end point combined to give good check determinations.

A. Alfend.—I checked a portion of the acid against Bureau of Standards acid potassium phthalate and against sulfuric acid made up determinately, through sodium hydroxide solution. The indicated strength was 0.1000 in each case. As for the silver chloride, I have no explanation to offer, nor do I have any confidence in the method.

J. P. Aumer.—No difficulty was experienced with the anhydrous sodium carbonate or the borax standardizations.

Jonas Carol.—No trouble was encountered in these standardizations. However, the borax method is apparently much easier to use, as the methyl red end point is much more definite than that obtained by using methyl orange.

E. H. Berry.— " . . . Of the two, I prefer borax, although equally accurate re-

[1] F. Hurley, *Ind. Eng. Chem. Anal. Ed.*, **8**, 220 (1936); **9**, 237 (1937).

[2] G. F. Smith and Croad, *Ibid.*, 141–42; Kolthoff and Furman, Volumetric Analysis, Vol. II, 87–93 (1929).

[3] *J. Am. Chem. Soc.*, **45**, 1223 (1923).

TABLE 1.—*Collaborative results on 0.1 N HCl solution. (All normalities are corrected to 20° C.)*

COLLABORATOR	PROCEDURE		
	SILVER CHLORIDE	SODIUM CARBONATE	BORAX
G. M. Johnson	0.09944	0.1000	0.1003
Minneapolis	0.09944	0.1001	0.1002
	0.09932	0.1002	0.1003
	0.09998	0.1001	0.1003
	0.09993		0.1003
			0.1003
Av.	0.09962	0.1001	0.1003
S. Alfend	0.0995	0.1003	0.1003
St. Louis	0.0995	0.1003	0.1002
	0.0996	0.1003	0.1002
Av.	0.0995	0.1003	0.1002
J. P. Aumer	0.1001	0.1002	0.1002
New Orleans	0.1001	0.1002	0.1002
		0.1002	0.1003
Av.	0.1001	0.1002	0.1002
J. T. Field[a]	0.0998	0.1000	0.1001
St. Louis	0.0997	0.1000	0.1002
Av.	0.09975	0.1000	0.10015
A. E. Plumb[a]	0.0996	0.1003	0.1002
St. Louis		0.1005	0.1004
Av.	0.0996	0.1004	0.1003
J. Carol	0.1000	0.1001	0.1000
Cincinnati	0.0999	0.1000	0.1001
	0.1000	0.0999	0.1001
Av.	0.1000	0.1000	0.1001
E. H. Berry	0.0999	0.1001	0.1001
Chicago	0.0999	0.1002	0.1001
Av.	0.0999	0.10015	0.1001
R. L. Vandaveer	0.0998	0.1001	0.1001
Chicago	0.0997	0.1000	0.1000
	0.0999	0.1000	0.1000
			0.1000
Av.	0.0998	0.1000	0.1000

[a] Used portions from same bottle.

sults were obtained with sodium carbonate. I believe that more accurate results can be obtained with either the borax or sodium carbonate than with the present silver chloride method, and would like to see standardization of acid solutions using these salts made official.

Table 2 summarizes the collaborative results based on each analyst's average normality value.

TABLE 2.—*Summary of Table 1 data based on each average result*

		METHOD		
		SILVER CHLORIDE	SODIUM CARBONATE	BORAX
Range of Collaborative Results { Max.		0.1001	0.1004	0.1003
{ Min.		0.0995	0.1000	0.1000
Average (Mean)		0.09978	0.10014	0.10017
Mean Error of Mean—(Dm)—		0.00007	0.00006	0.00004
corresponding to an accuracy of (%)		0.07	0 06	0.04
Av. Deviation		0.00017	0 0001	0.00008

I. Sodium Tetraborate

The commercial preparations of this salt are readily obtained pure, and the product may also be easily prepared pure by recrystallization from water. The salt neither loses nor gains moisture during weighing. The large equivalent weight, which practically eliminates weighing error for 0.1 N solutions, and the absence of indicator error at the equivalence point when methyl red indicator is used[1] make this compound an extremely useful primary standard for acidimetry. According to the experimental evidence accumulated by F. Hurley,[2] borax may be stored for lengthy periods without change, if placed in a desiccator over a solution saturated with respect to sucrose and salt. The Referee has kept a sample of borax under these conditions for six months with no measurable change in neutralizing power by checking the sample against a standard hydrochloric acid solution. This method of keeping borax in an atmosphere of proper humidity overcomes its tendency to lose some of its molecular water, which may occur if the salt is allowed to remain in dry air for more than five days. The method presented for the standardization of acid solutions was published in *This Journal*, 22, 102.

II. Anhydrous Sodium Carbonate

Here, as with borax, in order to obtain a salt of utmost purity, sodium bicarbonate (or sodium carbonate) is subjected to recrystallization, ignition at 290° C., and test for possible impurities before use. The sodium carbonate so prepared is a satisfactory primary standard for acid solu-

[1] Kolthoff and Furman, Volumetric Analysis, Vol. II, 95 (1929).
[2] *Ind. Eng. Chem. Anal. Ed.*, 9, 237–38 (1937).

tions with methyl orange indicator. R. A. Osborn[1] has noted that this salt, if not heated above 290° C., is not hydroscopic during the normal period of weighing in open air.

The temperature at which the salt is completely decomposed into normal carbonate has a definite bearing upon its ultimate composition. G. F. Smith and Croad[2] have experimentally demonstrated that above 300° C. the rate of decomposition of sodium carbonate is proportional to the temperature. No decomposition of the salt was found at 300° C.

The only disadvantage of sodium carbonate as a primary standard under normal conditions is that titrations must be performed in the acid range with methyl orange or methyl yellow. The ʃsubstance may be titrated to phenolphthalein if the carbon dioxide is removed by boiling. (NOTE: The working titer of the solution standardized is accurate only with the indicator with which the normality value was obtained. The correction to be applied may be determined. See *Methods of Analysis, A.O.A.C.*, 1935, 682.

The method presented for the standardization of acid solutions with sodium carbonate was published in *This Journal*, 22, 103.

III. Precipitation as AgCl[3]

This well-known procedure measures the acid strength indirectly; the chloride content is calculated to its acid equivalent. However, it was believed desirable to submit the procedure to collaborative study.

DISCUSSION OF RESULTS

In any set of collaborative results, given a sufficient number, there is likely to be some deviation from the true value. It becomes then a matter of deciding whether the results obtained are sufficiently close to the absolute concentration (if such is known) to determine the accuracy of a method. Also to be taken into consideration is the standardization error that might affect the practical result of a determination in which the standard solution may be used.[4]

The results obtained by both borax and sodium carbonate are in excellent agreement; those by silver chloride are materially lower. The greatest range of normalities determined by a single collaborator, in the case of borax and sodium carbonate, is two parts in a thousand; this is in contrast with the range of results of 0.6 per cent when the silver chloride method, as reported by one collaborator, was used.

If it is assumed that the solution, which was prepared from constant boiling HCl to be 0.1000 *N*, is such, then the collaborator whose determination with borax is farthest away from this value would be in error of no more than 3 parts in a thousand. With sodium carbonate the

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[1] Private communication.
[2] *Ind. Eng. Chem. Anal. Ed.*, 9, 141 (1937).
[3] *Methods of Analysis, A.O.A.C.*, 1935, 23.

greatest deviation is 4 parts in a thousand; while with silver chloride the maximum difference from 0.1 *N* is 0.5 per cent low.

CONCLUSIONS

On the basis of the collaborators' results and their comments, and because the silver chloride procedure has certain inherent errors due principally to solubility of silver chloride,[1] it is concluded that further study of silver chloride as a method for standardization is unwarranted.

With respect to standardization by means of borax and sodium carbonate, it is believed that these two well-recognized procedures will fill A.O.A.C. requirements for accurate and direct methods of assaying the strength of acid solutions.

It is recommended[2]—

(1) That the method for the standardization of acid solutions with borax be adopted as tentative.

(2) That the method for the standardization of acid solutions with sodium carbonate be adopted as tentative.

(3) That studies on the standardization of hydrochloric acid solutions by silver chloride be discontinued.

REPORT ON IODINE AND THIOSULFATE SOLUTIONS

By KENNETH L. MILSTEAD (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The work of the Associate Referee this year on the preparation and standardization of iodine and thiosulfate was concerned with a preliminary study of existing and well-known methods. The work was confined to—

(1) Preparation of standard iodine solution by direct weighing of purified iodine.
(2) Standardization of iodine solution against Bureau of Standards arsenious oxide.
(3) Standardization of thiosulfate solution against standard iodine solution.
(4) Standardization of thiosulfate against potassium iodate.

Preparation of Iodine Solution by Direct Weighing

A good grade of resublimed iodine was resublimed once from a mixture of potassium iodide and ignited lime and three times from ignited lime alone. A standard solution of iodine was prepared from the purified crystals as follows:

A large weighing bottle containing 40 grams of potassium iodide and 10 cc. of water was accurately weighed after the contents had come to room temperature. To the weighing bottle was added approximately

[1] Morey, *J. Am. Chem. Soc.*, **34**, 1039 (1912).
[2] For report of Subcommittee A and action by the Association, see *This Journal*, **22**, 49 (1939).

the amount of purified iodine required for 1000 cc. of a 0.1 *N* solution; the bottle was stoppered again and accurately weighed. The contents were transferred quantitatively to a 1000 cc. flask and made to the mark with water at 20° C. The actual weight of iodine added was 14.4130 grams. The calculated normality of this solution at 20° C. was 0.11356.

Standardization of Iodine Solution Against Arsenious Oxide

A standard arsenite solution was prepared with Bureau of Standards sample No. 83 and the method outlined in the Certificate of Analysis accompanying the standard sample; 4.5012 grams of arsenic trioxide was made to a volume of 1000 cc. at 20° C. The calculated normality of this solution was 0.90998 at 20° C.

Accurately measured portions of the standard arsenite solution were titrated with the iodine solution prepared by direct weighing following the Bureau of Standards directions. (The starch indicator used was prepared by triturating 2 grams of soluble starch and 10 mg. of mercuric iodide with a little water and adding the suspension to 1 liter of boiling water. The boiling was continued until the solution was clear; 5 cc. of this starch solution was used for each 100 cc. of solution to be titrated.) The results are given in Table 1.

TABLE 1.—*Standardization of iodine solution against arsenious oxide*

ARSENITE SOLN	TEMP.	ARSENITE SOLN 20° C.	IODINE SOLN	TEMP.	IODINE SOLN 20° C.	NORMALITY IODINE SOLN 20° C.	AV. NORMALITY BY ARSENITE SOLN	DEVIATION FROM MEAN	DIFF. FROM VALUE BY DIRECT WEIGHING
cc.	° C.	cc.	cc.	° C.	cc.	cc.			per cent
50	31	49.87	40.05	31	39.95	0.11358	0.11357	0.009	0.02
50	31	49.87	40.06	31	39.96	0.11355		0.02	0.01
50	31	49.87	40.03	31	39.93	0.11363		0.06	0.07
50	31	49.87	40.04	31	39.94	0.11361		0.04	0.05
50	31	49.87	40.08	31	39.98	0.11350		0.07	0.06
50	30	49.88	40.06	30	39.97	0.11354		0.03	0.02
50	30	49.88	40.04	30	39.95	0.11361		0.04	0.05
Av. Deviation								0.04	

The data indicate that the standardization of iodine solution by means of direct weighing and by the use of National Bureau of Standards standard sample of arsenious oxide No. 83 should agree within one part in 2000.

Standardization of Thiosulfate Against Standard Iodine Solution

The thiosulfate solution was prepared by dissolving approximately 25 grams of sodium thiosulfate in 1 liter of freshly boiled and cooled distilled water; 0.1 gram of sodium carbonate was added, and the solution was allowed to stand for 1 week before standardization.

Accurately measured portions of the iodine solution prepared by direct weighing and standardized against arsenious oxide were transferred to Erlenmeyer flasks, 5 cc. of 1 *N* sulfuric acid was added and the solution was titrated with thiosulfate solutions. Five cc. of starch indicator was added near the end point. The results are given in Table 2.

TABLE 2.—*Standardization of thiosulfate solution against standard iodine solution*

IODINE SOLN	TEMP.	IODINE SOLN 20° C.	THIOSULFATE SOLN	TEMP.	THIOSULFATE SOLN	NORMALITY THIOSULFATE 20° C.
cc.	° C.	cc.	cc.	° C.	cc.	
50.0	31	49.87	48.31	31	48.18	0.11756
50.0	31	49.87	48.33	31	48.20	0.11750
50.0	31	49.87	48.30	31	48.17	0.11757
50.0	31	49.87	48.30	31	48.17	0.11757
Av. normality thiosulfate against standard iodine						0.11755

Standardization of Thiosulfate Against Potassium Iodate

In this study attention was directed to the use of analytical grade potassium iodate without purification as a primary standard for thiosulfate because if offers many advantages over other substances that have been recommended for this purpose. Some attention was also given to the purification of potassium iodate by recrystallization from water, since it is reported in the literature that this procedure yields a pure product.

At the completion of the work on potassium iodate the thiosulfate solution being standardized was again titrated against the standard iodine. No change in the normality of the thiosulfate as determined against iodine solution occurred during the course of the work on potassium iodate.

TABLE 3.—*Standardization of thiosulfate against potassium iodate*

IODATE SOLN	TEMP.	IODATE SOLN 20° C.	THIOSULFATE SOLN	TEMP.	THIOSULFATE SOLN 20° C.	NORMALITY THIOSULFATE SOLN 20° C.	AV. NORMALITY BY IODATE	DIFF. FROM VALUE BY IODINE SOLN
cc.	° C.	cc.	cc.	° C.	cc.			per cent
50.0	31	49.87	42.90	31	42.77	0.11729	0.11728	0.2
50.0	31	49.87	42.90	31	42.77	0.11729		0.2
50.0	31	49.87	42.92	31	42.79	0.11723		0.3
50.0	31	49.87	42.90	31	42.77	0.11729		0.2
50.0	31	49.87	42.90	31	42.77	0.11729		0.2

Analytical grade of potassium iodate was dried at 180° C.; 3.5882 grams of the dried salt, weighed from a weighing bottle by difference, was transferred to a 1 liter volumetric flask and made to the mark with

water at 20° C. The calculated normality of this solution was 0.10059 at 20° C.

Accurately measured portions of this solution were transferred to Erlenmeyer flasks, 2 grams of potassium iodide (iodate-free) and 10 cc. of 1 N sulfuric acid were added, and the liberated iodine was titrated with thiosulfate. Five cc. of starch solution was added near the end point. The results are recorded in Table 3.

The results in Table 3 show that the normality of thiosulfate determined against unpurified analytical grade potassium iodate does not agree with the normality determined against iodine.

It would appear that the potassium iodate contains some impurity that liberates a larger amount of iodine than would be liberated by a corresponding amount of potassium iodate.

In an attempt to determine whether the discrepancy was due to an impurity in the iodate, a sample of the analytical grade was recrystallized from water twice and dried at 180° C.; 3.5025 grams of the purified and dried salt was dissolved in water and made to a liter at 20° C. The calculated normality of this solution was 0.09819 at 20° C. Accurately measured portions of this solution were titrated with the thiosulfate as previously described. The results are recorded in Table 4.

TABLE 4.—*Standardization of thiosulfate solution against purified potassium iodate*

IODATE SOLN	TEMP.	IODATE SOLN 20° C.	THIOSULFATE SOLN	TEMP.	THIOSULFATE 20° C.	NORMALITY THIOSULFATE 20° C.	DIFF. FROM VALUE BY IODINE
cc.	° C.	cc.	cc.	° C.	cc.		per cent
50.0	29.5	49.89	41.90	31	41.80	0.11720	0.3
50.0	29.5	49.89	41.90	31	41.80	0.11720	0.3
50.0	29.5	49.89	41.90	31	41.80	0.11720	0.3
Av. normality, by purified iodate						0.11720	

The results in Table 4 indicate that the impurity present in the potassium iodate is not eliminated by recrystallization from water, but that the purified salt contains a higher concentration of the impurity than the unpurified. This impurity is probably sodium iodate.

CONCLUSIONS

The normality of an iodine solution prepared by direct weighing of the purified iodine agrees closely with the normality determined by titration against Bureau of Standards arsenious oxide.

Analytical grade of potassium iodate is not a satisfactory primary standard for the standarization of thiosulfate, nor does recrystallization twice from water improve its quality for this purpose.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method outlined in the certificate accompanying Bureau of Standards standard sample No. 83 of arsenic trioxide for the standardization of 0.1 N solution of iodine be made tentative with the view to official adoption.

(2) That the standardization of thiosulfate be studied further.

The method follows:

IODINE

REAGENTS

(a) *Arsenic trioxide.*—U. S. Bureau of Standards sample. Dry 1 hour at 105° C. immediately before using.

(b) *Starch indicator.*—Triturate 2 grams of soluble starch and 10 mg. of HgI_2 (preservative) with a little H_2O and add the suspension slowly to 1 liter of boiling H_2O. Continue boiling until solution is clear. Use 5 cc. of this starch solution for each 100 cc. of solution to be titrated.

(c) *Arsenite solution.*—Approximately 0.1 N. Preferably weigh the sample by difference in a small weighing bottle owing to the difficulty of completely brushing As_2O_3 from metallic or glass surfaces. Accurately weigh a stoppered weighing bottle containing approximately 4.95 grams of As_2O_3. Transfer without loss to a graduated liter flask and again weigh the bottle. Do not attempt to brush out the adhering oxide. Moisten the sample with H_2O, add 15 grams of pure NaOH and 100 cc. of distilled H_2O. Swirl the contents of the flask gently until the As_2O_3 is in solution. Dilute to 250 cc. with H_2O and saturate the solution with CO_2, thus converting all the NaOH to $NaHCO_3$. Dilute to the mark, mix thoroly, and stopper the flask. A solution thus prepared will preserve its titer almost indefinitely. If the solution is made up on a volume basis make corrections afterwards for temperature changes.

(d) *Iodine solution.*—Approximately 0.1N. Dissolve 12.7 grams of resublimed I and 20 grams of pure KI in 50 cc. of H_2O. When the I has dissolved transfer the solution to a glass-stoppered liter flask, dilute to mark with H_2O, mix thoroly, and stopper the flask.

STANDARDIZATION

Transfer an accurately measured portion (40–50 cc.) of the arsenite solution to a flask and titrate with the 0.1N iodine solution, using starch solution as indicator. To obtain accurate results it is absolutely necessary that the solution be saturated with CO_2 at the end of the titration. A current of CO_2 may be passed through the solution for a few minutes just before the end point is reached, or a few drops of HCl may be added to liberate sufficient CO_2 to saturate the solution. If the flask is stoppered immediately after the completion of the titration, the pink or rose-colored end point is stable for days.

From the quantities of I and arsenite solutions used calculate the titer of the I solution on the basis of the following relation:

$$As_2O_3 + 2I_2 + 2H_2O \rightarrow As_2O_5 + 4HI.$$
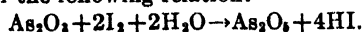
No report on insecticides, fungicides, and caustic poisons was given by the referee. —————

No report on fluorine compounds was given by the associate referee.

REPORT ON PYRETHRUM PRODUCTS, DERRIS, AND CUBE

By J. J. T. GRAHAM (Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.), *Associate Referee*

During 1938 this Associate Referee gave his attention to methods for the analysis of pyrethrum products, derris, and cube powder.

DERRIS AND CUBE POWDER

The collaborators were asked to determine the rotenone and total ether extract. For the rotenone determination Method 1, the chloroform extraction or crystallization method adopted last year as tentative, *This Journal*, 21, 148, and Method 2, the Jones titration method,[1] were used.

METHOD 2, TITRATION METHOD

Extract the root sample and crystallize the rotenone from CCl_4 at 0° C. as described by Jones and Graham.[2] Filter and wash the precipitate as usual, and without further drying dissolve it in about 25 cc. of acetone in a 250 cc. flask. (This is readily accomplished by placing the crucible in a funnel and washing the contents through into the flask with small quantities of acetone.) Evaporate the solvent on the steam bath and treat the residue with 10 cc. of 80% (by volume) dichloroacetic acid, warming gently until the residue is just dissolved. Cool the solution for a few minutes in a bath of ice water. Add 10 cc. of cold water slowly while swirling the flask. Add a few seed crystals of rotenone-dichloroacetic acid solvate and again cool the flask in ice water 2 or 3 minutes. (Separation of a few small needle crystals will usually be noted at this point.) If no crystals separate, add water a drop or two at a time with intermittent cooling until a few crystals are seen, then add water 10–15 drops at a time with about 1 minute cooling periods between additions until 25 cc. has been added. Add 25 cc. additional water dropwise and again cool the solution. Finally add 50 cc. more water at a faster rate and again cool the solution. Filter the material through a Gooch crucible fitted with a disk of filter paper and wash with about 250 cc. of water in small portions. (It is well to remove the crucible from the holder after several washings and wash the outside of the crucible and the rubber holder and then replace the crucible and dissolve the contents in 25 cc. of $CHCl_3$.) If preferred, the crucible and contents may be placed in a beaker, the $CHCl_3$ added to dissolve the contents, and the crucible left in the beaker during the titration. Add 50 cc. of freshly boiled water and titrate the mixture with 0.1 N alkali, using phenolphthalein or bromothymol blue as indicator. Thoroughly agitate the mixture, particularly near the end point, to insure that all the acid is extracted from the $CHCl_3$ layer.

Each cc. of 0.1 N alkali is equivalent to 39.4 mg. of rotenone. Make the usual allowances, mentioned in the method cited, for added rotenone and for solubility of rotenone in CCl_4 and run a blank on the $CHCl_3$ used.

The results of the collaborators are given in Table 1.

DISCUSSION OF METHODS

Method 1, the tentative chloroform extraction method, gave good results on both the derris and cube samples.

[1] *Ind. Eng. Chem. Anal. Ed.*, 10, 684 (1938).
[2] *Ibid.*, 19; *This Journal*, 21, 148 (1938).

TABLE 1.—*Collaborative results on derris and cube powder*

ANALYST	SAMPLE 1			SAMPLE 2		
	ROTENONE		ETHER EXTRACT	ROTENONE		ETHER EXTRACT
	METHOD 1	METHOD 2		METHOD 1	METHOD 2	
			per cent			
Analyst "A"	4.3		14.1	5.4		22.4
Sacramento	4.3			5.5		
Average	4.3			5.5		
Analyst "B"	4.3		14.0	5 4		22.0
Sacramento						
H. Bois		4.9	13.8		6.0	21.1
San Francisco		5.1	13 7		6.3	21.2
Average		5.0	13.8		6.2	21.2
C. G. Donovan	4.4			5 2		
Washington	4.4			5.2		
	4.4			5.4		
Average	4.4			5.3		
—. —. Feelemeyer	3.8		13.9	5.0		21.3
Baltimore	3.9		13.7	5.0		21.7
			14.0			21.8
			14.0			21.5
Average	3.9		13.9	5 0		21.6
J. J. T. Graham	4.6	4.0	14.5	5.7	5.3	23.3
	4.5	4.1		5.8	5.3	
	4.5	4.4		5.8	5.3	
		4.3		5.6	5.5	
		4.5				
Average	4.5	4.3		5.7	5.4	
D. G. Hoyer	4.4	4.7	13.8	5.5		21.4
New York	4.2	5.3	13.8	5.4		21.4
	4.5		13.8	5.7		21.4
Average	4.4	5.0	13.8	5.5		21.4
H. A. Jones	4.3			5.3		
Washington	4.6			5 2		
Average	4.5			5.3		
R. D. Stanley	4.1		14.1	5.1		22.2
Chicago	3.9		14.1	5.1		
Average	4.0		14.1	5.1		
A. Wolf	4.4			5.4		
Washington	4.7			5.4		
Average	4.6			5.4		

Method 2 is a new method developed by H. A. Jones. It is based on a sound principle and has given good results in the hands of Jones and his co-workers but several of the collaborating analysts had difficulty with it. One analyst commented that it appeared difficult, if not impossible, to wash out excess acid from such a sticky, gummy mass as that obtained in preparing the dichloracetic acid derivative. Another analyst reported that he obtained one or two results that checked with the crystallization method, but that subsequent assays gave results with no consistency in any direction. The rotenone acetic acid solvate forms small needle crystals that filter readily when properly precipitated. Apparently these analysts did not obtain the rotenone in this form. It is important that sufficient time be allowed between the additions of water during the crystallization to allow the crystals to form, otherwise the rotenone and impurities will be thrown down as an amorphous mass and incorrect results obtained. The method should receive further study.

PYRETHRUM PRODUCTS

Two samples were prepared for collaborative testing of the methods for the determination of pyrethrins. Sample 5 was a good grade of commercial pyrethrum powder. Sample 4 was a mineral oil extract prepared by dissolving the residue from a petroleum ether extract in a highly refined mineral oil base. To this was added, by volume oil of birch 1.5 per cent, Optone (a commercial preparation of rotenone in essential oils for incorporation in fly sprays) 1 per cent, and Lethane 384, 3 per cent. This sample had a theoretical pyrethrin content of 0.053 per cent calculated from the Associate Referee's analysis of the mineral oil-pyrethrum extract used in its preparation. These samples were sent to the collaborators with the following directions for analysis:

Pyrethrum Powder

PYRETHRIN I

Method 1.—(Seil, *Soap*, **10**, May 1934, pp. 89, 91, 111.)

Extract 12.5 grams of flowers ground to 30 mesh, or finer, in a Soxhlet extractor for 7 hours with petroleum ether (boiling range 30–60°). Evaporate the ether on a water bath, heating no longer than necessary to remove all the solvent. Add to the residue 20 cc. of 0.5 N alcoholic NaOH solution and boil gently under a reflux condenser 1–2 hours. Transfer the alkaline alcoholic solution to a 600 cc. beaker, washing the flask with water. Add sufficient water to bring the volume of liquid in the beaker to 200 cc. Add a few glass beads or introduce a boiling tube and boil to remove alcohol, using care to avoid boiling over due to saponification. When the volume has been reduced to 150 cc., cool and transfer to a 250 cc. volumetric flask to which has been added 1 gram of filter-cel. Add 10 cc. of 10% $BaCl_2$ solution, make to volume, and thoroughly mix the solution by shaking. Filter through a fluted paper, transfer 200 cc. of the filtrate to a 500 cc. flask, add 5 cc. of H_2SO_4 (1+4), and distil, using a distillation trap and an efficient condenser, until the volume has been reduced to about 20–30 cc. Put a low flame under the flask and pass steam through until 300–350 cc. of distillate has been collected. (The flask

should rest upon a piece of asbestos board in which a hole has been cut of sufficient size to allow the contents to be heated without super heating the sides of the flask above the liquid.) Receive the distillate in a 500 cc. separatory funnel. At this point the separatory funnel will contain the mono-carboxylic acid and the distillation flask the di-carboxylic acid.

To the separatory funnel add 50 cc. of petroleum ether and shake thoroughly. After the liquids have separated, draw off the aqueous layer into a second separatory funnel and again extract with 50 cc. of petroleum ether and discard the aqueous layer. Wash the extracts in the two funnels successively with 10 cc. of water and discard the water after it has passed through the second funnel. Wash again with another 10 cc. of water and discard as before. Combine the petroleum ether extracts. Neutralize 15 cc. of water containing 1 or 2 drops of phenolphthalein indicator solution with $0.02 N$ NaOH solution, add to it the combined petroleum ether solutions, and titrate with $0.02N$ NaOH solution, shaking after each addition, until the aqueous layer is just pink. Each cc. of $0.02 N$ NaOH solution consumed is equal to 0.0066 gram of Pyrethrin I.

Method 2.—Proceed as directed under the mercury reduction method, *This Journal*, 21, 78 (1938).

PYRETHRIN II

Method 1

Allow the residue from the steam distillation to cool and filter through a Gooch crucible, washing the flask with a little water. Make the clear filtrate alkaline with $NaHCO_3$ transfer to a separatory funnel, and wash twice with $CHCl_3$. Wash the $CHCl_3$ extracts successively through one wash water of 10–15 cc. and combine the aqueous solutions. Acidify with about 10 cc. of HCl and saturate with salt, adding very cautiously at first to avoid excessive ebullition of CO_2. Extract with 50 cc. of ether, shaking for about 1 minute. Draw off the aqueous layer into a second funnel and again extract with 50 cc. of ether. Continue the extractions in a third and a fourth funnel, using in each case 35 cc. of ether. Each time that the aqueous solutions or washings are drawn off from the separatory funnels use care to see that the droplets adhering to the walls are removed as completely as possible. Wash the four ether extracts successively with two 10 cc. portions of water and then combine the ether solutions. Tap off any water that separates and filter the ether solution into a 500 cc. Erlenmeyer flask. Evaporate the ether on a water bath and dry the residue at 100° for 10 minutes. Add 2 cc. of neutral alcohol, warm gently, add 20 cc. of water, and heat to dissolve the acid. If a residue remains undissolved, cool and filter through a Gooch crucible. Add 1 or 2 drops of phenolphthalein indicator solution and titrate with $0.02 N$ NaOH solution. One cc. of $0.02 N$ NaOH solution is equivalent to 0.00374 gram of Pyrethrin II.

Method 2

Filter the aqueous residue from the petroleum ether extraction in the determination of Pyrethrin I by Method 2 (mercury reduction), through a Gooch crucible. Concentrate the filtrate to about 50 cc., transfer to a separatory funnel and proceed as directed in Method 1, beginning with "make alkaline with $NaHCO_3$."

PYRETHRUM EXTRACTS IN MINERAL OIL

PYRETHRIN I

Method 1. (Seil method modified.)

Transfer 100 cc. of the ordinary household extract, or less quantity of a concentrate, into a 500 cc. flask. If a volume less than 100 cc. is used, make up to that

volume with a highly refined mineral oil of the type used for the base of ordinary household extracts. Distil with steam until about 500 cc. has passed over, which should remove the perfume. Do not put a flame under the flask containing the sample during this distillation. Discard the distillate and transfer the residue in the flask to a 500 cc. separatory funnel, rinsing the flask with small portions of kerosene. Drain off the aqueous layer into another separatory funnel and wash with 25 cc. of a highly refined mineral oil. (It may be necessary to add a little saturated salt solution to get a clean separation. It may also be necessary to wash with several portions of the mineral oil to get complete separation of the oil-soluble portion from the aqueous solution.)

Transfer the oil containing the pyrethrins to an Erlenmeyer flask, add 20 cc. (or more if necessary) of 0.5 N alcoholic NaOH solution, and reflux 1–2 hours on a hot plate, using a vertical condenser.

Transfer the alkaline solution to a 600–800 cc. beaker. Wash the flask with water, adding the washings to the solution. Add sufficient water to bring the aqueous layer to 200 cc. or more if additional alcoholic NaOH has been used, and boil gently until the aqueous layer is reduced to 150 cc. to remove alcohol. Keep a boiling rod or glass beads in the beaker to prevent bumping. Cool, transfer to a separatory funnel and draw off the oil-free portion of the lower layer into a 250 cc. volumetric flask. Wash out the beaker with small additional quantities of water, adding the washings to the separatory funnel, mixing the contents, and drawing off the separated aqueous layers between washings. Finally, if some emulsion still persists, add a few drops of 10% BaCl$_2$ solution to break the remaining emulsion. Do not shake after adding the BaCl$_2$ to the separatory-funnel, because the reversed emulsion that may be formed cannot readily be broken. Discard the kerosene. Add 1 gram of filter-cel to the volumetric flask, then add 10 cc. of BaCl$_2$ solution, and shake thoroughly until the solution is clear, adding more BaCl$_2$ solution if necessary. When the solid material is coagulated, fill the flask to the mark, mix, and filter the solution through a fluted paper. Transfer 200 cc. of the filtrate to the flask of a steam distillation apparatus, add 5 cc. of H$_2$SO$_4$ (1+4), and proceed as directed under "Pyrethrum Powder, Method 1," beginning with the steam distillation following the addition of the dilute H$_2$SO$_4$.

PYRETHRIN II

Method 1.—Proceed as directed under Pyrethrum Powder, Method 1.

Method 2.—Proceed as directed in the mercury reduction method, *This Journal,* 21, 78.

The results of the collaborators are given in Table 2.

DISCUSSION OF METHODS

On the pyrethrum powder, Sample 5, both methods gave results that agree fairly closely in most cases, although some variations are too great. On the mineral oil-pyrethrum extract, Method 2 (mercury reduction) gave results for Pyrethrin I that were more consistent than those by Method 1, the Seil method. The results for Pyrethrin II on the mineral oil-pyrethrum extract show a rather wide variation probably due to incomplete removal of the esters in the preliminary steam distillation. The collaborative work is insufficient to justify any recommendation other than that the work on pyrethrum products be continued.

Ripert[1] suggested that mineral oil-pyrethrum extracts should have free

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[1] *Ann. fals.*, 27, 577 (1934).

TABLE 2.—*Collaborative results on pyrethrum products*

| ANALYST | SAMPLE 4 | | | SAMPLE 5 | | | |
| | METHOD 1 PYRETHRIN | | METHOD 2 PYRETHRIN | METHOD 1 PYRETHRIN | | METHOD 2 PYRETHRIN | |
	I	II	I	I	II	I	II
			per cent				
J. A. Batscha	.073			.33	.34		
New York	.071			.32	.35		
	.072			.30	.41		
				.29	.38		
Average	.072			.31	.37		
J. C. Bubb	.066			.37	.47		
New York	.076	.25		.33	.45		
Average	.071			.35	.46		
C. G. Donovan			.049	.26	.41	.25	.41
J. J. T. Graham	.055	.094	.055	.28	.47	.27	.43
	.051	.094	.055	.28	.50	.27	.46
			.054			.27	
			.056			.27	
Average	.053	.094	.055	.28	.49	.27	.45
D. G. Hoyer	.069	.37		.26	.40	.21	.39
	.067	.18		.26	.40	.21	.42
	.062	.10					
Average	.066	.22		.26	.40	.21	.41
A. Wolf			.061				
			.063				
Average			.062				
H. Bois	.052	.069	.048	.26	.37	.21	.39
	.050	.076	.049	.26	.39	.22	.37
Average	.051	.073	.049	.26	.38	.22	.38

acids removed with aqueous normal alkali before the saponification with alcoholic alkali. Martin[1] applied this procedure to the petroleum ether solution in the analysis of pyrethrum flowers. Alvin J. Cox of the California Department of Agriculture has suggested the use of 50 per cent aqueous sodium hydroxide for extraction of acidic substances from commercial mineral oil-pyrethrum insecticides.

Wilcoxon[2] and also Pantsios[3] have shown that there is a heavy loss of

[1] *J. Agr. Sci.*, **28**, 456 (1938).
[2] *Contr. Boyce Thompson Inst.*, **8**, 175 (1936).
[3] *Ind. Eng. Chem. Anal. Ed.*, **10**, 386 (1935).

Pyrethrin I during the distillation of the acid solution in the Seil method. Martin investigated this loss and found that it depends largely upon the quantity of excess sulfuric acid present. He also found that by neutralizing the solution and then adding 1 cc. of normal acid in excess the results obtained check those obtained by the Wilcoxon mercury reduction method.

RECOMMENDATIONS[1]

It is recommended—

(1) That the study of methods for the analysis of derris and cube products be continued, and that special attention be given to the chloroform extraction and the Jones titration methods.

(2) That the study of methods of analysis of pyrethrum products be continued, with special attention given to the effect of a preliminary treatment for the removal of acidic substances, and also to a study of the effect of varying quantities of excess sulfuric acid on the decomposition of the pyrethrins during the distillation.

No report on naphthalene in poultry lice products was given by the associate referee.

No report on disinfectants was given by the referee.

REPORT ON SUGARS AND SUGAR PRODUCTS

By RICHARD F. JACKSON (National Bureau of Standards, Washington, D. C.), Referee

The Referee has studied in detail the methods of analysis of sugar products and is impressed by the care and assiduous attention to detail that have been exercised by previous referees and associate referees. The methods are well presented and in general reflect fairly present knowledge of the subject. Some comments on current problems suggest themselves.

For the analysis of reducing sugars it is probable that in spite of the great number of modifications that have been described most analyses are made by Munson and Walker's method. While many modifications are more rapid and convenient, there are but few that approach it in respect to precision. The comprehensiveness of the tables and the fact that the personal equation is not an important factor have added to its advantages. In view of its prevalence it has seemed important to verify the copper-sugar equivalents. Such an investigation has been completed by Dr. L. D. Hammond at the National Bureau of Standards and will be available for the next edition of Methods of Analysis. Dr. Hammond has determined reduced copper by electrolysis. A similar research has been

partially completed by the Referee and Dr. Emma J. McDonald, who have determinationed copper by volumetric analysis.

An extension of Munson and Walker's table is highly to be recommended. There are two columns of copper-sugar equivalents adapted to sucrose-invert sugar mixtures in which 0.4 and 2.0 grams of total sugar, respectively, are taken for the analysis. These permit the determination of reducing sugars in mixtures in which a maximum of 60 per cent of the total sugar is reducing sugar. Above this maximum there are no tabulated data that are applicable, and the analyst is forced to the doubtful expedient of interpolating between pure invert sugar and the 0.4 gram total sugar column. Again at several intermediate ratios of invert sugar to total sugar, analysis is possible only by taking quantities of sample that reduce small weights of copper. Thus, of a mixture containing 13 per cent invert sugar and 87 per cent sucrose, a 0.4 gram sample containing 52 mg. of reducing sugar must be taken for analysis. This mixture can of course be analyzed, but with far less precision than a sample four times greater.

This situation will be partially rectified in Dr. Hammond's new table, but further extension of the principle is desirable. If a systematic series of experiments were made in which the sucrose were varied within very wide limits, it would be possible to construct tables or formulas for the evaluation of the effect of sucrose and thus increase greatly the flexibility of the method. Such a system would make it possible to replace the cumbersome Meissl and Hiller method by one based upon modern analyses. These investigations are in progress in the laboratories of the National Bureau of Standards.

The modern tendency has been to devise modifications of reducing sugar methods in which the entire analysis is completed in a single reaction vessel by volumetric procedure. Such a method is that of Lane and Eynon, in which the sugar solution is added to the point of complete reduction of the copper. In other modifications a portion of the copper is reduced under standard conditions and the reduced or unreduced copper determined by supplemental titration.

A method of this class was described by Shaffer and Hartmann, who suggested two modes of procedure, the one adapted to relatively large samples (200 mg. or less), the other to small samples (2 mg. or less). The latter has been extensively studied, particularly in its application to biological fluids. It has proved equally applicable to general chemical problems and the Referee believes it would serve a useful purpose in the analysis of materials with which this Association is concerned when it is necessary to analyze small samples. The macro method was not elaborated by Shaffer and Hartmann, but has been studied and modified by the Referee and Dr. McDonald. It is believed that both the Shaffer and Hartmann methods could advantageously be included.

The Clerget method, which in the present year has reached the ninetieth anniversary of its inception, is still beset with numerous problems, many of which are of a fundamental nature. In order that the method might rest upon a firm basis of accurately determined constants and correction coefficients, the Referee and Dr. McDonald have remeasured with care the basic values of the Clerget divisor under varied conditions of inversion, and the corrections to be applied for varied concentration and temperature. These experiments are described in a separate paper, which follows.

THE BASIC VALUES OF THE CLERGET DIVISOR AND THE CORRECTION COEFFICIENTS

By RICHARD F. JACKSON and EMMA J. McDONALD

(National Bureau of Standards, Washington, D. C.)

The present discussion is concerned with the basic values of the Clerget divisor that obtain under the conditions of Herzfeld's modification of the Clerget method. In devising the modification Herzfeld measured the direct polarization in the usual manner by taking the normal weight of pure sucrose in 100 cc. and observing its rotation in a 200 mm. column at 20° C. This rotation is by definition 100° S. For the invert polarization he took the half-normal weight (13 grams) in 75 cc. of solution, added 5 cc. of hydrochloric acid (38 per cent, or sp.gr. 1.188), immersed the flask in a water bath, and within 2 to 3 minutes warmed the contents to 67°–70° C. The solution having attained the temperature of the bath, he kept it as near 69° C. as possible for another 5 minutes, when it was quickly cooled, made to a volume of 100 cc. at 20° C., and polarized at the same temperature. Being that of a half-normal solution, the reading was multiplied by 2. Under these conditions the invert polarization was found by Dammüller[1] to be −32.66 at 20° C.

Later investigators have invariably obtained higher values; thus Tolman[2] found −32.88; Steuerwald[3] −33.05, Walker[4] −32.78, and Jackson and Gillis[5] a computed value of −33.08.

In 1920 Herzfeld published a posthumous work of Schrefeld, whose experiments were made in 1912. Schrefeld followed Herzfeld's procedure but defined the conditions of hydrolysis more precisely by specifying that a thermometer be inserted in the solution, which was agitated in a water bath at 70° C. until in 2½–2¾ minutes it had reached a temperature of 67°. From this moment he allowed it to remain in the bath for exactly 5 minutes, during which time the temperature gradually rose to 69.5°,

[1] *Z. Ver. deut. Zucker-Ind.*, 38, 699, 746 (1888).
[2] *U. S. Dept. Agr. Bur. Chem. Bull.*, 73, 73 (1903).
[3] *Int. Sugar J.*, 16, 82 (1914).
[4] *Sugar*, 17, No. 2, 47 (1915).
[5] *Sci. Pap. BS*, 16, 153 (1920), 8375.

whereupon it was rapidly cooled to 20°, diluted to 100 cc., and polarized after standing for at least $\frac{1}{2}$ hour. As a mean of seven concordant measurements Schrefeld found the invert polarization to be −33.00. This value has been verified by Browne and Gamble.[1] Zerban and his co-workers[2] found −32.97, and Spengler, Zablinsky, and Wolf[3] −33.02. In a single measurement the present writers found the value −32.99. The mean polarization, −33.00, must be considered a well established value.

Jackson and Gillis showed that under the conditions of hydrolysis an appreciable destruction of invert sugar occurred. The writers have therefore sought to determine what the rotation would be if such destruction were avoided by shortening the final period of heating from 5 minutes by 1-minute intervals until a maximum negative rotation was reached. This maximum was attained by a 2-minute period of heating after the temperature of the solution reached 67° C., the reading being −33.08.

In 1920 Jackson and Gillis[4] published the results of a careful series of measurements made with solutions inverted in a bath at 60° C. instead of 70° C., the purpose being to avoid the destruction of invert sugar. Under these conditions they found the value −33.25. In order to eliminate instrument errors, however, they made their measurements on solutions more concentrated than 13 grams of sucrose in 100 cc. by comparing their rotations with those of such standard quartz plates as were available, and were thus obliged to correct their observations for the excess concentration, using the prevailing coefficient 0.0676 per gram of sugar in excess of 13 grams. Recent measurements discussed later show that this coefficient is too low and that a recalculation of their results is necessary. The recalculated value becomes −33.18.

The Arrhenius equation—

$$k_{T_1} = k_{T_2} e^{\frac{Q}{R}\left(\frac{T_2 - T_1}{T_1 T_2}\right)}.$$

in which k is the velocity constant at the absolute temperature, T, and Q/R the "activation energy," permits the calculation of the velocity constant at any desired temperature if the velocity at any one temperature and the constant, Q/R, are known. Jackson and Gillis[5] determined that in the presence of 0.7925 N hydrochloric acid, k_{30} was 0.002161 (common logs and minutes) and Q/R 13087.6. With the aid of this equation the writers calculated the time required for 99.99 per cent hydrolysis at 49° and at 35° C., respectively, and found for both temperatures the value −33.25 for twice the rotation of the half-normal solution.

[1] *J. Ind. Eng. Chem.*, 13, 793 (1921).
[2] Unpublished report to International Commission for Uniform Methods of Sugar Analysis.
[3] *Z. Wirtschaftsgruppe Zuckerind.*, 86, 670 (1936).
[4] *Sci. Pap. BS*, 16, 125 (1920), 8375.
[5] *Ibid.*, 132.

Many analysts advocate inversion at room temperature as a safe means of avoiding the decomposition of invert sugar. The Arrhenius equation enables the analyst to calculate the velocities of inversion and times required for 99.99 per cent completion of the reaction at the temperatures that may be expected in uncontrolled laboratories. These periods of time vary considerably with small changes of temperature, as is shown in Table 1.

TABLE 1.—*Time of hydrolysis of cane sugar at room temperature*

TEMPERATURE	k (COMMON LOG, MINUTES)	TIME FOR 99.99 PER CENT INVERSION	TEMPERATURE	k (COMMON LOG, MINUTES)	TIME FOR 99.99 PER CENT INVERSION
°C.		*hours*	°C.		*hours*
18	0.001590	41.9	26	0.005290	12.6
20	0.002161	30.8	28	0.007073	9.4
22	0.002924	22.8	30	0.009421	7.08
24	0.003941	16.9	32	0.012502	5.33

While the velocities of many reactions double themselves with a rise of 10° in temperature, the velocity of inversion of cane sugar increases more than fourfold between 20° and 30°. Thus room temperature is safe only if such variations in temperature as inevitably occur are known to the analyst and are considered in calculating the time required for complete hydrolysis. It appears from Table 1 that 24 hours is insufficient for hydrolysis at 20° and that 16 or 17 hours for overnight inversion at 30° is excessive and serious decomposition of invert sugar can result. Evidently room temperature inversion must be carried out with considerable discretion.

For room temperature inversion the value of the negative constituent of the Clerget divisor adopted by this Association is −33.20. The writers measured this constant with care by inverting the half-normal solution for the calculated time in a thermostat that maintained a constant temperature within a few hundredths of a degree and observing its rotation at 20° C. The mean value found was −33.29 for solutions inverted at 25° C.

In recapitulation, Table 2 shows the values obtained under the conditions described in the previous paragraphs. These conditions, in all determinations except the first, were so adjusted that no decomposition of invert sugar occurred after the completion of the inversion. It is evident that the rotation is definitely a function of the temperature at which the sugar is inverted and that invert sugar is attacked by acid during the course of the inversion. This destructive reaction is apparently not the same in kind as that of acid upon ordinary invert sugar, for in additional experiments not tabulated the value remained unchanged when the time of heating at 60° C. was prolonged to 13 minutes. Conceivably furanoid

fructose, which has a transitory existence, is attacked by the acid the more strongly the higher the temperature.

TABLE 2.—*Variation of the negative constituent of the Clerget divisor with varied conditions of inversion*

TEMPERATURE OF INVERSION	TIME	ROTATION×2 AT 20° C.
°C.	*minutes*	°S.
67–69	5.0	−33.00
67–69	2.0	−33.08
60	9.5	−33.18
49	38 0	−33.25
35	205.0	−33.25
25	17.2 hrs.	−33.29

CONCENTRATION COEFFICIENT OF THE CLERGET DIVISOR

The specific rotation of both dextrose and levulose varies with the concentration of sugar, and that of invert sugar likewise varies with concentration, as is shown by Gubbe's[1] equation, $[\alpha]_D^{20} = -19.447 - 0.06068p + 0.000221\ p^2$, in which p is the per cent invert sugar. Thus the basic values discussed previously are valid only for a concentration of 13 grams of inverted sucrose in 100 cc.

Herzfeld applied to the basic value the correction $0.0676\ (m-13)$, in which m is the weight of inverted sucrose in 100 cc. of the solution taken for the invert polarization. This value of the coefficient has remained in general use to the present day, although Steuerwald found the slightly higher value 0.0717, Herles 0.067, and Sazavsky 0.0677. Zerban in his reports to the International Commission for Uniform Methods of Sugar Analysis has repeatedly recommended that both the concentration and temperature coefficients be reinvestigated.

The solutions for the determination of the concentration coefficient were prepared by dissolving 65 grams of pure sucrose in 310 cc. of water, or 130 grams in 269.2 cc., the total volume being in each case 350 cc. To each of these was added 50 cc. of hydrochloric acid (d 20/4 = 1.1029), and the sugar was inverted in a thermostat at 20°, or at 23.7° C. for the period of time calculated by means of the Arrhenius equation. The solutions were made to a volume of 500 cc. at 20° C., carefully weighed, and polarized at 20° C. Aliquot portions were then transferred to 100 cc. flasks, weighed, and made to volume after addition of such quantities of acid that each contained 10 cc. per 100 cc. All these solutions were polarized at 20°C. By this procedure assurance was had that all variables such as those arising from the inversion reaction itself were eliminated, the only

[1] *Ber.*, **18**, 2207 (1885)

variable left being that caused by dilution. All readings were calculated to 26 grams of sucrose. The rotations were found by graphic plot and by mathematical analysis to diminish linearly with concentration for the entire range of concentrations between 26 and about 5 grams of sucrose. The values obtained in four series of measurements, adjusted by the method of least squares, yielded, respectively, the equations

$$P' = -(32.244 + 0.0783 \ s)$$
$$= -(32.245 + 0.0803 \ s)$$
$$= -(32.265 + 0.0794 \ s)$$
$$= -(32.259 + 0.0794 \ s)$$
$$\text{Mean} \ \ P' = -(32.253 + 0.07935 \ s) \tag{1}$$

in which s equals the grams of sucrose. The coefficient, 0.07935, is surprisingly higher than hitherto reported.

At the lower concentrations of sugar the small errors of observation are multiplied many fold in the calculation to 26 grams of sucrose. Hence a complete series of measurements was made by dilution of an acidified stock solution containing but 7 grams of sucrose in 100 cc. A least-square adjustment yielded the equation—

$$P' = -(32.243 + 0.0834 \ s). \tag{2}$$

The slightly higher coefficient indicates that a slightly increased slope occurs at very low concentrations, but the difference is within the experimental error of measurement. A solution of equations (1) and (2) yields identical values for 5 grams of sucrose and a difference for 2 grams corresponding to a difference in observation of 0.001° S. (200 mm.). For practical purposes Equation (1) can be extended to all concentrations of sugar below 26 grams.

The concentration coefficients just described were measured on solutions that contained 10 cc. of 6.34 N hydrochloric acid in accordance with the specifications of the "acid" Clerget method. It is now of interest to determine similar coefficients of invert sugar solutions in the absence of acid, since such is the condition in the enzyme Clerget method.

Invert sugar solutions were prepared by weighing out equal weights of very pure dextrose and levulose, the methods of purification of which are described elsewhere. A stock solution (500 cc.) was prepared containing 67.3702 grams each of pure dextrose and levulose, corresponding to 128.0032 grams of sucrose. The sugars were dissolved and made to volume after the completion of the mutarotation. As in the previous experiments, aliquot portions were taken, weighed, and made to volume. The solutions were allowed to stand overnight in a thermostat at 20° C. before polarization. All measurements were calculated to 26 grams of sucrose. A least-square adjustment yielded the equation—

$$P' = -(30.994 + 0.08241 \ s) \tag{3}$$

the relation being linear between 26 and 7 grams of sucrose.

Two typical series are given in Table 3. If the formula for the acidified solution is solved for 13 grams of sucrose, the divisor becomes -33.29, in agreement with the direct determination described previously. If that for the synthetic invert sugar is similarly solved, the divisor becomes -32.07, which is but slightly lower than Paine and Balch's value, -32.11, for invertase inversion. The difference between -33.29 and -32.07, or $1.22°$, is due to the presence of 10 cc. of acid. Jackson and Gillis by a somewhat different method found 1.25.

TABLE 3.—*Typical measurements of the concentration coefficient of invert sugar*

10 cc. 6.34 N HCl $P' = -(32.253+0.07935\ s)$			SYNTHETIC INVERT SUGAR $P' = -(30.994+0.08241\ s)$		
WEIGHT OF SUCROSE	P' FOUND	P' CALCULATED	WEIGHT OF SUCROSE	P' FOUND	P' CALCULATED
grams			*grams*		
26.000	34.33	34.33	25.6001	33.11	33.10
23.6201	34.13	34.14	23.2542	32.91	32.91
20.7796	33 93	33.91	20.4571	32.66	32.68
18 1639	33.68	33.70	17.8814	32 47	32.47
15.5841	33.51	33 50	15.3376	32.25	32.26
12.9891	33.29	33.29	12.7940	32.08	32.05
10.6115	33.13	33 11	10.1919	31.85	32.84
7.7942	32.84	32.89	7.6688	31.61	31 63
5.1782	32.71	32.68	—	—	—

It is now of interest to calculate the value of the concentration coefficient that Gubbe's specific rotation equation would yield. Since in this formula concentrations are expressed as percentages, the calculation of concentration by volume requires a knowledge of the densities of the respective solutions. These densities are not well established, but if two solutions are selected differing by only 1 per cent concentration, say 13 and 14 per cent, the analyst is dealing merely with differences in concentration and any errors in absolute magnitudes are largely eliminated. It is then necessary to convert from circular degrees of sodium light to saccharimeter degrees with white light, and the conversion factor, 34.615, determined directly by Zerban,[1] is available for this purpose. The calculation carried out in this manner yielded the value 0.083 between 13 and 14 grams of sucrose, in good agreement with the directly determined coefficient of the writers, 0.0824. Between 26 and 27 grams of sucrose Gubbe's coefficient becomes 0.0687, which is in less satisfactory agreement.

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[1] *J. Am. Chem. Soc.*, **47**, 1110 (1925)

Zerban[1] has calculated from analyses previously published[2] the coefficient for the range of 6.5 to 13 grams of sucrose, finding 0.0831, and between 13 and 26 grams, 0.0781.

Thus the available data indicate that the higher value of the concentration coefficient is more nearly correct than the value, 0.0676, in present use.

A few experiments, preliminary in nature, were made to determine the temperature coefficient of the Clerget divisor. The coefficient that has been in use since Clerget devised the method is 0.5 t, although some doubt in respect to its validity has been expressed, particularly when it is applied to crude products.

The invert sugar solutions used by the present writers were prepared in much the same manner as those for the concentration coefficient. From 500 cc. of a weighed solution containing 143.279 grams of sucrose that had been inverted with 50 cc. of hydrochloric acid (d 20/4 1.1029) at 20° C., aliquot portions were transferred to 100 cc. flasks and weighed. These portions were so selected that the final solutions contained four concentrations of sugar, each in duplicate. Sufficient additional acid was added so that each solution contained 10 cc. One set of the four solutions was made to volume and polarized at 20° C., the other at 25° or 30° C., and polarized in a 400 mm. column in a thermostated room at the same temperature. The most reliable set of observations was obtained in a series polarized at 20° C. and at 30° C. One writer observed a mean coefficient of 0.4960, the other of 0.4963. For an interval of 10° this would make the corrected divisor 0.04° higher than would the coefficient 0.5, or about 0.03 per cent. Additional measurements in both acid and neutral solutions are in progress.

In summary, the present investigation has tended to show (1) that the negative constituent of the Clerget divisor in acid solution is a function of the temperature of inversion, (2) that the value of the divisor for room temperature inversion should be 133.29 instead of 133.20 at 20° C., (3) that the Jackson-Gillis value of the divisor for inversion at 60° C. should be 133.18 instead of 133.25, (4) that the concentration coefficient in acid solution should be 0.0794 instead of 0.0676, (5) that the concentration coefficient in neutral solution should be 0.0824, and (6) as a preliminary conclusion, that the temperature coefficient, 0.5 t, is very nearly correct, at least for pure invert sugar.

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No report on honey was given by the associate referee.

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No report on maple products was given by the associate referee.

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[1] Private communication.
[2] *This Journal*, **8**, 384 (1925).

REPORT ON DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS

By CARL F. SNYDER (National Bureau of Standards, Washington, D. C.), *Associate Referee*

It is recommended*—

(1) That the International Scale of Refractive Indices of Sucrose Solutions at 20° C., 1936, be adopted as official (final action).

(2) That the International Temperature Correction Table, 1936, be adopted as official (final action).

(3) That the vacuum drying method for the determination of moisture in cane and beet raw and refined sugars be adopted as official (final action), *This Journal* 21, 89 (1938).

(4) That the direct drying method, *Methods of Analysis*, A.O.A.C., 1935, 462, 2, now official, be made a tentative method.

No report on polariscopic methods (general) was given by the associate referee.

No report on chemical methods for reducing sugars was given by the associate referee.

REPORT ON ACETYLMETHYLCARBINOL AND DIACETYL IN FOOD PRODUCTS

By JOHN B. WILSON (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

Since last year's report was submitted, the attention of the Associate Referee has been directed to an article by Hooft and Leeuw,[1] who show that both acetylmethylcarbinol and diacetyl occur in commercial bread. The method used in the analytical work was the familiar precipitation as nickel dimethylglyoxime and a special type of apparatus was used to prevent loss of diacetyl.

The new colorimetric method of Pien, Baisse, and Martin[2] mentioned in last year's report, *This Journal*, 21, 427, has now become available to the Associate Referee since after considerable difficulty a quantity of the reagent, diaminobenzidene, has been obtained.

Another colorimetric procedure based on the formation of a ferric iron-dimethylglyoxime complex is now recommended by Prill and Hammer.[3]

During the year some experimental work on determination of diacetyl

* For report of Subcommittee D and action by the Association, see *This Journal*, 22, 64 (1939).
[1] *Cereal Chem.*, 12, 213 (1935).
[2] *Lait*, 17, 673 (1937).
[3] *Iowa State Coll. J. Science*, 12, 385 (1938).

was carried out, diacetylmonoxine and diacetyldioxime (dimethylgly-oxime) being used as the source of diacetyl, but no conclusion has as yet been reached as to the best procedure for quantities of diacetyl greater than 10 mg.

It is intended during the coming year to make a comparative study of the two outstanding colorimetric methods mentioned above, both of which are highly recommended by those who have investigated them.

It is recommended that investigations on methods for determination of acetylmethylcarbinol and diacetyl be continued.

No report on unfermentable sugars in molasses was given by the associate referee.

No report on refractive indices of sugar solutions was given by the associate referee.

REPORT ON VINEGARS

By A. M. HENRY (U. S. Food and Drug Administration,
Atlanta, Ga.), *Referee*

It is recommended*—

(1) That the method proposed by the Associate Referee for the determination of ash in vinegar, *This Journal*, 21, 89, and adopted as official (first action) last year, be adopted as official (final action).

(2) That methods for the determination of total phosphoric acid be studied.

(3) That the official method for the determination of solids in vinegar be studied, especially with reference to its applicability to vinegars high in solids, such as malt vinegar.

(4) That methods for the detection of caramel in vinegar be continued.

(5) That methods for the determination of dilute acetic acid in vinegar be studied.

PRELIMINARY INVESTIGATION OF THE LICHTHARDT TEST FOR THE DETECTION OF CARAMEL IN CIDER AND DISTILLED VINEGARS

By FRANK E. COOK and HARVEY MILLER (Eighth Corps Area
General Depot, Inspection Laboratory,
Fort Sam Houston, Texas)

The Lichthardt test[1] was applied to various pure cider and distilled vinegars, to which had been added solutions of caramel from various

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* For report of Subcommittee C and action by the Association, see *This Journal*, 22, 63 (1939).
[1] *J. Ind. Eng. Chem.*, 2, 389 (1910).

sources. The writers desire to thank Mr. Etter of the San Antonio Brewing Co., and Mr. Fetzer of the Union Starch and Refining Co., for the samples of caramel coloring which they so kindly furnished.

The Lichthardt test follows:

Dissolve 1 gram of tannic acid in 30 cc. of water. Add 0.75 gram of H_2SO_4 (sp.g. 1.84). (A precipitate formed on the first addition of H_2SO_4 will dissolve.) Make up the volume to 50 cc. with water, let stand 24 hours, and filter. The reagent keeps well. To 5 cc. of vinegar add 5 cc. of reagent. Heat the mixture gently until the precipitate at first formed is nearly dissolved. Set aside 12 hours (overnight). A brown precipitate adhering closely to the bottom of the tube indicates caramel, and a flocculent, non-adhering precipitate is to be ignored.

An initial examination of the Lichthardt tannic acid reagent showed that the volume produced by the formula would be insufficient for any series of tests, and also that it is difficult to weigh concentrated sulfuric acid but easy to measure it.

The following formula, which it is believed keeps the original characteristics of the reagent, was used:

Add 4 cc. of H_2SO_4 (sp.g. 1.84) to 9.8 grams of tannic acid dissolved in 294 cc. of water. Make to 490 cc. with water. (The H_2SO_4 was added from a 4 cc. pipet, and the pipet was washed to remove the remaining acid.)

The first tests showed that often no precipitate was formed on the addition of the reagent to the vinegar, whether it contained caramel or not. This raised the question of how much heat, if any, should be applied. Various degrees of heating were tried, as shown in Table 2. Except for the above noted changes, the method used was as originally outlined.

TABLE 1.—*Analyses of vinegars used*

TESTS	I	II	III	IV	V	VI	VII	VIII
Acidity (g./100 cc. as acetic)	5.16	6.18	4.74	4.00	5.07	4.05	4.40	4.05
Total Solids (g./100 cc.)	1.45	2.47	1.74		1.47	1.48	1.75	2.10
Ash (g./100 cc.)	0.28	0.32	0.28		0.27	0.47	0.31	0.29
Alkalinity of water-soluble ash $\frac{cc.\ 0.1\ N\ Acid}{100\ cc.}$	28.0	30.0	26.0		24.0	48 0	27.0	27.0
Qualitative Tests								
Sulfates	trace	trace	trace		trace	trace	trace	trace
Calcium	trace	trace	trace		trace	trace	trace	trace
Chlorides	none	none	none		none	present	none	none
Ppt. w/neutral Pb(AC)	O.K.	O.K.	O.K.		O.K.	slight	O.K.	O.K.

TABLE 2.—*Effect of varying conditions on caramels and vinegars*

KIND OF VINEGAR	CONDI- TION	SOURCE OF CARAMEL							
		0	1	2	3	4	5	6	7
I	a	neg. 3* sl. ppt. 1	pos.	pos.	pos.	pos. 2			
	b	neg. 1	pos. 2		pos. 2	pos. 2			
	c		pos.		pos.	pos.			
	d	neg. sl. ppt. 1	pos.		pos.	pos.			
	e-40	neg. 1 sl. ppt. 1	pos.		pos.	pos. 2	pos.	pos.	
	e-44	neg. 1 sl. ppt. 1	pos.		pos.	pos. 2	pos.	pos.	
	e-48	neg. 1 sl. ppt. 1	pos.		pos.	pos. 2	pos.	pos.	
II	a	neg. 2	pos.	pos.	pos.	poor 2 settling			
III	a	neg.	pos.	pos.	pos.	pos.			
IV	a	pos. 3 sl. ppt. 3	(Colored distilled vinegar)			poor 2 settling			
V	a	sl. ppt. 2							
	b		pos. 2		pos. 2	pos. 2			
	e-40	sl. ppt. 2			pos.	pos.	pos.	pos.	
	e-44	sl. ppt. 2			pos.	pos.	pos.	pos.	
	e-48	sl. ppt. 2			pos.	pos.	pos.	pos.	
VI	a	neg. 2				cloudy 2			
	b	neg. 3	pos. 3		pos. 3	pos. 3			
	c		pos.		pos.	cloudy			
	d		pos.		cloudy	cloudy			
	e-40	neg. 2 sl. ppt. 1	pos.		pos.	pos. 2	pos.	pos.	
	e-44	neg. 2	pos.		pos.	pos. 2	pos.	pos.	pos. 2
	e-48	neg. 2	pos.		pos.	pos. 2	pos.	pos.	
VII	a	neg. 2				cloudy			
	b	neg. 3	pos. 3		pos. 3	pos. 3			
	c		pos.		pos.	cloudy			
	d	neg.	pos.		pos.	cloudy			
	e-40	neg. 2	pos.		pos.	pos. 2	pos.	pos.	
	e-44	neg. 3	pos.		pos.	pos. 2	pos.	pos.	pos. 2
	e-48	neg. 2	pos.		pos.	pos. 2	pos.	pos.	
VIII	a	neg. 2				cloudy			
	b	neg. 3	pos. 3		pos. 3	pos. 3			
	c		pos.		pos.	cloudy			
	d		pos.		pos.	cloudy			
	e-40	neg. 2	pos.		pos.	pos. 2	pos.	pos.	
	e-44	neg. 3	pos.		pos.	pos. 2	pos.	pos.	pos. 2
	e-48	neg. 2	pos.		pos.	pos. 2	pos.	pos.	
Water & H(AC)	a	neg. 2			pos. 4	pos. 4			
	e-44	neg.							pos. 2

The A.O.A.C. modification of Amthor's test for caramel was made on vinegar No. V, as this vinegar gave a slight precipitate under all conditions when the Lichthardt test was applied. The results were unsatisfactory and inconclusive.

RESULTS

(1) Some caramels gave no immediate precipitate when the tannic acid reagent was added.

(2) Uncontrolled heating of the vinegars and caramel with the reagent gave unsatisfactory results.

(3) Temperatures of 50° C. and above interfere with the formation and settling of the precipitate.

(4) Temperatures up to 48° C. held for not over 4 minutes do not interfere with the formation and settling of the precipitate.

(5) Vinegars Nos. I and VI, after standing in the laboratory for 3 to 4 months, and being opened repeatedly, commenced to show slight precipitates with the tannic acid reagent, where previously they had shown none.

(6) Vinegars Nos. I and VI also showed a marked progressive darkening in color on standing through the 3–4 month period.

(7) Vinegar No. 5 showed a slight precipitate with the tannic acid reagent under all conditions of test.

(8) The precipitate termed "positive," when allowed to form unmolested while settling, would spread itself over the side walls and bottom of the test tube, but a slight twist of the tube would cause it to settle to the bottom.

(9) No precipitate was noted actually to adhere to the side walls or bottom of the tube.

(10) Very little difference was noted in the nature of the precipitate obtained from vinegars containing added caramel and those which had no caramel added.

DISCUSSION

The vinegars were the unsolicited samples that were "on hand" at

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* Source of caramel and designation:
0.—No caramel, 1.—Imitation maple flavoring, 2.—Fictitious vanilla extract, 3 —Imitation vanilla extract, 4.—Caramel malt coloring (from San Antonio Brewing Co.), 5.—Caramel Coloring (Drug Store), 6.—Caramel—Acid Proof—"Pennant" (Union Starch & Refining Co.), 7.— Laboratory-prepared caramel. The caramels from the sources designated as 1, 2, and 3 were added to the vinegars in the proportion of 1 cc. of solution to 4 cc. of vinegar. The caramel concentrates designated as 4, 5, 6, and 7 were first diluted to approximately 1:200 with water and then added to the vinegar in the proportion to 1 cc. of diluted caramel to 4 cc. of vinegar.
Subscripts indicate number of tests run.
Negative (neg.) indicates no precipitate formed.
Positive (pos.) indicates more than slight precipitate.
Sl. ppt. indicates slight precipitate.
Conditions under which tests were run:
(a) Heated—no specific control (as no precipitate formed)
(b) Not heated—room temperature (30°–40° C.).
(c) Heated to 50° C., held for 5 minutes, removed, cooled in air.
(d) Heated to boiling, removed, cooled in air.
(e) Placed in water held at 40, 44, and 48° C., respectively, for 4 minutes, removed, cooled in air.

the time of this investigation. They were all, with the exception of No. IV (colored distilled), considered to be pure cider.

Vinegars I, II, III, and IV showed expected reactions under varying conditions, and were considered normal.

Vinegars V, VI, VII, and VIII failed to give precipitates under all conditions, and were, therefore, selected for the remaining tests because of their erratic behavior.

Vinegar I was considered representative of the first group and was also used in the remaining tests, however it also turned out to be erratic in that it finally gave precipitates when no caramel was present.

Vinegars I and VI were very light in color when received. On standing in the laboratory in the presence of (among other things) sunlight and air, a very marked progressive darkening occurred. As received, these vinegars gave no precipitate with Lichthardt's tannic acid reagent, but after standing 3–4 months both gave slight precipitates.

The original instructions directed that gentle heat be applied until the precipitate that forms is nearly dissolved. Caramel coloring No. 5 was the only caramel, or vinegar, that gave an immediate noticeable precipitate and to which the original instructions were applicable. An attempt was made to obtain selective precipitation through control of the heating. This was only partially successful as some vinegars gave a slight precipitate when no caramel was present.

Temperatures of 40–48° C. for 4 minutes did not inhibit precipitation, as the higher temperatures did. Although these temperatures were used for the majority of the tests, they showed no specific advantage over room temperature (30–40° C.)

The precipitate produced when caramel had been added to the vinegars was slightly less flocculent and less easily disturbed from the sides and bottom of the tube than the precipitate produced when there was no caramel present. There was, of course, less precipitate when no caramel was present.

CONCLUSIONS

1. The difficulty of this method lies in the differentiation between the types of precipitates obtained from pure vinegars and those from vinegars containing added caramel.

2. Some vinegars will develop bodies on standing (perhaps caramel-like), which will give a precipitate with Lichthardt's tannic acid reagent.

3. It may be the import of the original method to apply no heat to the vinegar and tannic acid reagent unless a precipitate is immediately formed.

4. The method shows sufficient promise to warrant further investigation into the conditions under which the precipitates formed can be readily differentiated.

No report on ash in vinegar was given by the associate referee.

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By John B. Wilson (U. S. Food and Drug Administration,
Washington, D. C.), *General Referee*

No collaborative work was done on this subject during the past year. However, at this meeting the Referee is presenting papers on a quick method for determination of coumarin in imitation vanilla, and a method for determination of beta-ionone, which are worthy of collaborative study. See *This Journal*, 22, 378–96.

In reviewing the methods contained in the chapter on flavoring extracts in *Methods of Analysis, A.O.A.C.,* 1935, the thought occurred to the Referee that several of the methods in current use might be subjected to modern improvements to bring them up to date in regard to the type of apparatus used. For example, an automatic extractor might be applied to the gravimetric determination of vanillin and coumarin, and the spectrophotometer might be applied in the methods for the colorimetric determination of vanillin, aldehydes, citral, etc.

This year the associate referee appointed on the general subject of organic solvents has successfully investigated a chemical method for the determination of isopropyl alcohol. The Referee concurs in the associate referee's recommendation that the proposed method be further studied collaboratively.

RECOMMENDATIONS

It is recommended[1]—

(1) That the proposed chemical method for determination of isopropyl alcohol be studied further collaboratively.

(2) That the proposed chemical method for determination of isopropyl alcohol be applied to mixtures containing essential oils.

(3) That the photometric method for coumarin in imitation vanilla be subjected to collaborative study.

(4) That the proposed method for quantitative determination of beta-ionone be studied collaboratively.

(5) That the Referee study the advantages of the automatic extraction of vanillin and coumarin.

(6) That the Referee study the application of the photometer to the present colorimetric methods in the chapters on Flavors and Non-alcoholic Beverages.

REPORT ON ORGANIC SOLVENTS IN FLAVORS

By R. D. STANLEY (U. S. Food and Drug Administration,
Chicago, Ill.), *Associate Referee*

A search was made of manufacturers' catalogs and booklets to determine what solvents are available and the uses for which they are recommended. Basing his conclusions partly on this search and eliminating those unlikely to appear in foods or drugs and those low-boiling products used principally for extraction with later elimination of the solvent, the Associate Referee decided that the most important solvents likely to be found in food or drug products are water soluble and include ethyl and isopropyl alcohol, acetone, glycerol, the glycols, and glycol-ethers. The water-soluble solvents are considered of first importance since the most general use of an organic solvent in food and drug products, and particularly in flavors, is to hold in solution with water those materials otherwise insoluble. Some also are of importance because of their poisonous nature, for example diethylene glycol, which was found in elixir sulfanilamide and which has also been used in some flavors.

Isopropyl alcohol has been found in flavors, in drugs, and in insecticides. There is no method for its estimation among the methods of the A.O.A.C., in the United States Pharmacopoeia or in the National Formulary. Therefore it was decided that methods for the determination of isopropyl alcohol should be studied.

Adams and Nicholls[1] determined isopropyl alcohol colorimetrically after oxidation to acetone; and Cassar[2] determined it in the presence of acetone by oxidation with dichromate and titration of the excess dichromate with sodium thiosulfate. Estimation by density and refractive index measurements has been recommended and the literature contains the tables of Lebo,[3] of Bennett and Garret,[4] and those recently compiled by Batsche and Reznek of the U. S. Food and Drug Administration, *This Journal*, **20**, 107.

An ideal method would provide for the estimation of isopropyl alcohol in the presence of any or all of those compounds that might be present in the distillate of the usual alcohol determination, and could include volatile water-soluble alcohols, aldehydes, and ketones. Density and refractometric procedures would not be applicable in such a mixture. Oxidation, wherein the isopropyl alcohol is oxidized to acetone, and aldehydes and primary alcohols to the corresponding acids, and determination of the acetone formed seems to be the method of choice. Such a method was devised by Stanley and Vandaveer[5] of the U. S. Food and Drug Ad-

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[1] *Analyst*, **54**, 2 (1929).
[2] *Ind. Eng. Chem.*, **19**, 1060 (1927).
[3] *J. Am. Chem. Soc.*, **43**, 1006 (1921).
[4] *Perfumery and Essential Oil Record*, **16**, 18 (1925
[5] Unpublished.

ministration in 1935, and was used by them in official work, but this method is not applicable if both acetone and isopropyl alcohol are present.

Experimental work this year was limited to isopropyl-alcohol, ethyl-alcohol, acetone, and water mixtures. The results are encouraging, and it is believed that the method proposed can be extended to commercial samples containing isopropyl alcohol if appropriate extraction and distillation procedures are supplied. Further work along this line is planned.

The method involves oxidation with dichromate, distillation, and determination of the acetone formed by the procedure given in U.S.P.XI for acetone. A qualitative test is made for acetone in the original sample, and if present it is removed with paraformaldehyde.[1] The methods used follow:

QUALITATIVE TEST FOR ACETONE

Distil a portion of the sample and collect the first 2 cc. Add 5 cc. of an alcoholic solution of o-nitrobenzaldehyde (5%), and 1 cc. of NaOH solution (10%). Mix, then shake with a small quantity of $CHCl_3$. A blue color in the $CHCl_3$ shows the presence of acetone.

ISOPROPYL ALCOHOL IN WATER MIXTURES

(Use foil-wrapped stoppers)

I. Acetone Present.—Place 1.5 grams of paraformaldehyde in a 200 cc. volumetric flask and add about 20 cc. of normal NaOH solution. Pipet an aliquot containing not over 0.8 gram of alcohol into the flask. (An approximation to the alcoholic content may be had by a specific gravity determination and reference to the ethyl alcohol tables.) Dilute the mixture with water to about 100 cc. Connect the flask to a reflux condenser and heat slowly on a hot plate just to boiling. Remove the hot plate, wash down the condenser, and when cool make to the mark and mix. Filter through a dry filter, if necessary pouring the solution back through the filter until a clear filtrate is obtained. Pipet 100 cc. into a 500 cc. Erlenmeyer flask, add 5 grams of $K_2Cr_2O_7$, and when most of the salt has dissolved, add 100 cc. of H_2SO_4 (1+3). Stopper the flask, swirl, and let stand 30 minutes. Add 100 cc. of $FeSO_4$ solution (25%). Connect the flask to a vertical condenser through a foam trap. Slowly distil about 100 cc. into a 500 cc. volumetric flask containing 200–300 cc. of cold water. Dilute to the mark, mix and pipet 25 cc. into a glass-stoppered flask containing 25 cc. of normal NaOH; add 50 cc. of standard $0.1N$ I_2 solution while swirling the flask. Allow to stand 15 minutes. Add 26 cc. of normal HCl and at once titrate the residual I_2 with standard $0.1N$ $Na_2S_2O_3$ solution, adding starch solution when the I_2 color is nearly discharged. Each cc. of $0.1N$ I_2 consumed in the reaction corresponds to 0.001001 gram of isopropyl alcohol ($CH_3CHOHCH_3$).

II. Acetone Absent.—Into a 500 cc. Erlenmeyer flask containing 50 cc. of approximately $2N$ $K_2Cr_2O_7$, pipet an aliquot containing not over 0.8 gram of alcohol. Dilute to about 100 cc. with H_2O and continue as directed previously, beginning with "Add 100 cc. of H_2SO_4 (1+3). . . ."

EXPERIMENTAL WORK

Anhydrous isopropyl alcohol was prepared by refluxing 500 cc. of reagent-grade isopropyl alcohol labeled 98% with 200 grams of lime for 1.5 hours. The alcohol was then distilled. Constants found were: Density

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[1] *Analyst,* **58**, 749 (1933).

(20/4) 0.7846, boiling point 82.2° C., refractive index (20° C.) 1.3774. The acetone was dried over calcium chloride and distilled. Reagent "absolute" ethyl was used.

The samples were prepared by weighing the alcohols and acetone in small glass-stoppered flasks and transferring with water to 100 cc. or 200 cc. volumetric flasks. Suitable aliquots were measured out for analysis, and the following results were obtained:

WEIGHED			FOUND	RECOVERY
ISOPROPYL ALCOHOL	ETHYL ALCOHOL	ACETONE	ISOPROPYL ALCOHOL	
grams	*grams*	*grams*	*grams*	*per cent*
3.7474			3.7413	99.8
3.7474			3.7163	99.2
3.0877			3.1070	100.6
3.1533	3.1710		3.1740	100.6
3.1533	3.1710	0.8 cc.*	3.1460	99.8
4.5612	4.0953	4.4666	4.5680	100.1

* 0.8 cc. acetone added to aliquot taken for analysis.

It is recommended[1] that the method submitted be studied further and extended to include isopropyl alcohol in mixtures containing essential oils, and that the methods be submitted to collaborative study.

REPORT ON MEAT AND MEAT PRODUCTS

By R. H. KERR (U. S. Bureau of Animal Industry, Washington, D. C.), *Referee*

A collaborative study was made of the method for nitrates in meat and meat food products, which was previously studied in 1937. Consideration was also given to the methods for coagulable nitrogen in meat and for copper in gelatin. The method for coagulable nitrogen has been criticized, in that directions for the use of the indicator are such as are likely to lead to interference when the filtrate is subsequently used for the determination of creatin. A clarifying change is recommended. The methods for copper and zinc in gelatin are not satisfactory for the quantities of those metals ordinarily occurring in commercial gelatin of good quality. Substitution of the methods used in the Bureau of Animal Industry laboratories for many years is recommended.

For the study of the nitrate method a mixture containing a known quantity of sodium nitrate was prepared and distributed to the collaborators, with instructions to mix thoroughly one gram of the mixture with 200 grams each of finely comminuted, uncooked, fresh meat, of

[1] For report of Subcommittee D and action by the Association, see *This Journal*, 22, 67 (1939).

smoked and cooked sausage, such as bologna or frankfurter style, and of canned corned beef, and to determine nitrates in each mixture in accordance with the method studied.

Reports were received from six collaborators, and the results are shown in the table.

Collaborative results

ANALYST BUREAU OF ANIMAL INDUSTRY	W. C. MCVEY, WASHINGTON	I. M. MYERS, OMAHA	W. B. FROMER, OMAHA	F. B. HILTY, CHICAGO	W. F. SCHROEDER CHICAGO	A. E. GRAHAM, SAN FRANCISCO
I. Test sample mixed with fresh meat NaNO₃, p.p.m.—						
Found in mixture	565	525	550	506	536	600
	580	525	550			530
Found in meat	30	28	26	trace	trace	53
	30	19	23			
Amount recovered	535	497	524	506	536	547
	530	506	527			477
II. Test sample mixed with bologna style sausage NaNO₃, p.p.m.—						
Found in mixture	890	743	796	532	552	795
	860	743	796			719
Found in sausage	360	240	240	30	30	267
	345	240	240			
Amount recovered	530	503	556	502	522	528
	515	503	556			452
III. Test sample mixed with canned corned beef NaNO₃, p.p.m.—						
Found in mixture	730	721	721	598	604	719
	720	728	721			682
Found in meat	180	228	228	75	80	227
	165	228	228			189
Amount recovered	550	493	493	523	524	492
	555	493	493			493

An analysis of the original mixture showed it to contain 10.2 per cent sodium nitrate. One gram added to 200 grams of meat was, therefore, equivalent to 510 p.p.m. in the mixture. The maximum quantity found by any analyst was 556 p.p.m., or 109 per cent of the amount added. The minimum quantity found was 452 p.p.m., corresponding to 90 per cent of the quantity added. Average of all determinations reported was 515 p.p.m., or 100.9 per cent of the quantity added. The variation in results includes all discrepancies due to possible lack of uniformity in the nitrate mixture distributed as well as imperfect mixing of the sample

with the meat, in addition to analytical discrepancies. The results appear to warrant adoption of the method as a tentative method in place of the phenolsulfonic acid method.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for nitrates studied be adopted as a tentative method. This method was published in *This Journal*, **22, 82**.

(2) That the phenoldisulfonic method, Chapter *XXVIII*, **14** and **15**, be dropped.

(3) That the words, "using the indicator outside the solution to avoid subsequent interference with the determination of creatin," under **31**, be added immediately following the words "neutralizing to phenolphthalein," in **28**, XXVIII.

(4) That paragraphs **64** and **65**, XXVIII, be dropped and the methods submitted by the Referee be substituted. (These methods were published in *This Journal*, **22, 84**.)

(5) That the words, "neutralize with 10 per cent NaOH solution," occurring in the last sentence of paragraph **18** be changed to read, "neutralize with NaOH solution (1+1)."

REPORT ON SPICES

ASSAY OF SAGE

By J. F. CLEVENGER (U. S. Food and Drug Administration, New York, N. Y.), *Referee*

Work was continued in accordance with the recommendations approved last year. W. H. Headley of the H. J. Heinz Company, Pittsburgh, Pa., Bernard J. Thiegs of the North Dakota Regulatory Department, Bismarck, N. D., and John Molitor of this Station collaborated.

Samples of coarsely ground Dalmatian sage stored in air-tight glass containers were given to the collaborators, who were directed to assay the sample by the method as outlined in *Method of Analysis, A.O.A.C.*, 1935, pp. 447–49.

Results of assay

	Clevenger	Headley	Theigs	Molitor
Volatile oil (cc. per 100 grams)	1.6	1.45	1.45	1.55
Specific gravity (25°/25°)	0.923	0.922	0.922	0.9225
Optical rotation*	+12.11	+11.7	+12.1	+13.6
Refractive index (20° C.)	1.464	1.464	1.461	1.462
Acid number	1.22	1.25	1.3	1.3
Ester number	16.24	16.45	16.8	24.3

* Angular degrees 25° C., 100 mm. tube, white light.

The results reported are considered satisfactory. Variations are prob-

ably accounted for, in part, by the different periods of time elapsing between grinding and assaying the sage.

It is recommended that this method,[1] which is now tentative, be made official, first action.

REPORT ON BAKING POWDERS

CREAM OF TARTAR AND TARTARIC ACID IN TARTATE BAKING POWDERS

By B. G. HARTMANN (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The methods for the determination of cream of tartar and tartaric acid in tartrate baking powders, described in *This Journal*, **13**, 386, were subjected to collaborative study.

Four members of Food Division, U. S. Food and Drug Administration, Washington, D. C., participated in the work: R. A. Osborn, V. E. Munsey, W. O. Winkler, and B. G. Hartmann. With the exception of the Referee, none of these analysts had had previous experience with the method.

The two samples submitted were prepared in the laboratory and had the following compositions: Sample No. 1 contained 44.9 per cent cream of tartar, and 5.5 per cent free tartaric acid; Sample No. 2 contained 59.86 per cent cream of tartar, and no free tartaric acid. The samples were carefully checked for tartaric acid, and the cream of tartar and tartaric acid used were purified by recrystallization.

METHODS

The texts of the methods submitted to the collaborators were changed from those given in the original paper. The revised methods were published in *This Journal*, **22**, 74.

The results follow:

Collaborative results

COLLABO-RATOR	SAMPLE 1			SAMPLE 2			SAMPLE 1	SAMPLE 2
	INDIRECT METHOD			INDIRECT METHOD			DIRECT METHOD	
	TOTAL TARTARIC (41.4)	CREAM OF TARTAR (44.9)	FREE TARTARIC (5.5)	TOTAL TARTARIC (47.9)	CREAM OF TARTAR (59.9)	FREE TARTARIC (0)	FREE TARTARIC (5.5)	FREE TARTARIC (0)
	per cent	*per cent*	*per cent* 5.1	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	41.5	46.0	4.5	46.8	58.1	0.5	5.3	0.1
2	41.5	44.6	5.9	47.1	57.9	0.9	5.4	0.2
3	42.1	45.9	5.5	48.3	58.4	1.6	5.3	0.0
4	41.4	43.9	6.4	47.6	58.1	1.2	5.4	0.3

[1] For report of Subcommittee C and action by the Association, see *This Journal*, **22**, 63 (1939).

DISCUSSION OF RESULTS

The method for the determination of cream of tartar and total tartaric acid is satisfactory. The indirect method for free tartaric acid is not reliable, particularly when applied to powders containing no free tartaric acid, and therefore its use for determining this constituent cannot be recommended. The direct method for free tartaric acid is accurate. An error of −3.5 per cent on a mixture containing approximately 50 per cent of cream of tartar cannot be considered serious.

No adverse criticisms were received from any one of the collaborators; the general opinion was that the methods are simple and rapid.

It is recommended[1] that the methods here discussed be adopted as official, first action.

No report on fish and other marine products was given by the referee.

The informal discussion by Fred Hillig on volatile acids as an approach to the evaluation of spoilage in canned fish will not be published.

REPORT ON CACAO PRODUCTS

By W. O. WINKLER (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

Study of the pectic acid method for shell was continued, and some work was done on the determination of milk protein.

PECTIC ACID METHOD FOR SHELL

It was indicated last year that the method for separating the gums from the pectin was not entirely satisfactory. A simple way of more effectively accomplishing this separation was found this year. The new procedure, which will take the place of the partial precipitation of the gums, proteins, etc. (which were extracted with the ammonium oxalate solution) with tannic acid is based upon the observation that these materials did not readily dissolve after dehydration on the steam bath. The new procedure following the precipitation of the pectins, etc., with 80 per cent acidified alcohol is as follows:

Dissolve, and with hot water from a wash bottle transfer the precipitate back to 800 cc. beaker from the centrifuge bottle. Avoid an excess of water. Add a few drops of phenolphthalein and then concentrated NH₄OH dropwise with stirring until the solution has a basic reaction. Stir if any undissolved gelatinous material remains. Add dropwise with stirring until the pink color disappears a 10% solution of citric acid and then add a few drops in excess. Evaporate the sample to about 20 cc. on a hot plate (to speed up evaporation) and then to dryness on a steam bath. Allow the beaker to remain on the bath at least 15 minutes after the sample appears

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 74 (1939).

completely dry; remove from the steam bath and add 70 cc. of cold distilled water. Stir with a policeman to remove the material from the bottom of the beaker and let stand 15 minutes with frequent stirring.

Filter off the insoluble matter on a 7 cm. Büchner funnel in a Büchner flask (500 cc. or smaller), using a filter paper overlain with paper pulp about $\frac{1}{16}-\frac{1}{8}$ inch thick. Wash the beaker and filter with two or three 15 cc. portions of water, washing out any residue. Transfer the filtrate to a 800 cc. beaker and dilute to 190–200 cc.

Cool the solution below 25° C. and proceed with the saponification and the precipitation of the calcium pectate, etc.

Experiments with the procedure showed that commercial fruit pectins previously dissolved and precipitated redissolved completely after the dehydration treatment. The shell extract also dissolved almost completely, and the portions that did not dissolve were in all probability not pectin. On the other hand, a large portion of the material extracted from nibs and precipitated by alcohol did not redissolve after the treatment. Moreover, separations of the calcium precipitate and "pectic acid" precipitate, which follow, were clean and sharp, and filtered without clogging or gumming.

PECTIC ACID BY CALCIUM PECTATE AND ACIDIFICATION VS. A.O.A.C. PECTIC ACID

The fact that the material resulting from this method is the same as that from the A.O.A.C. procedure for pectic acid in fruits is shown by the analysis of two commercial citrus pectins. The results are given in Table 1.

TABLE 1.—*Pectic acid in commercial citrus pectins*

PECTIN NO.	SAMPLE	BY SHELL METHOD		BY A.O.A.C. METHOD	
	mg.	*mg.*	*per cent*	*mg.*	*per cent*
1	200	83.2	41.6	82.6	41.3
2	200	62.0	31.0	61.1	30.5

The results (Table 1) indicate that the methods determine the same thing, namely, di-galacturonic acid.

INDICATIONS OF SHELL DURING DETERMINATION

There is often an indication of the presence of shell in a chocolate sample at the beginning of the determination. Following the centrifuging in the extractions with ether and acid alcohol, there is usually a considerable amount of darker and coarser material at the bottom of the bottle. The higher layers of the cacao mass are lighter in color and finer in division. The darker and coarser material in samples containing appreciable quantities of shell is more pronounced and can usually be easily recognized by comparison with samples that are practically shell-free. This

fact is generally observed in authentic samples of liquor of known shell content and in commercial samples giving a high pectic acid figure.

This observation suggested the possibility of further increasing the sensitivity of the method, or any method, by separating the top layers of the sample and using the bottom ones for the determination. In the case of a sample containing only a small amount of shell in which the figure obtained was due primarily to the liquor itself, the figure obtained on the lower layers would not be materially increased. However, on a sample containing appreciable amounts of shell, the majority of this would be found in the lower layers and the figure for pectic acid on this portion would be correspondingly increased. A fairly good separation of the lower layers might possibly be effected by the use of the proper shaped centrifuge bottle or tube.

PECTIC ACID IN AUTHENTIC CACAO SAMPLES

The results of pectic acid determinations on a number of authentic chocolate liquors (commercially prepared) are given in Table 2. The shell content of the samples was ascertained by hand picking the nibs used in their manufacture and by the addition, to such liquors, of known quantities of shell.

TABLE 2.—*Pectic acid in chocolate liquors*

SAMPLE NO.	SHELL, HAND PICKED (fat-free basis)	SHELL ADDED (fat-free basis)	PECTIC ACID (fat-free dry basis)
	per cent	*per cent*	*per cent*
1	0.49	0	0.11
2	0.91	0	0.14
3	0.45		0.10
4	0.95	0	0.16
5	0.95	4.0	0.32
6	0.95	9.85	0.66
7	0.95	14.2	0.87
8		100	5.18, 4.92

The results in Table 2 show that the method will readily distinguish the samples containing varying amounts of shell. The same thing is indicated in the analysis of a number of commercial cocoas. The samples represent the varieties of cocoas on the local market. The results are given in Table 3, and the various kinds of cocoa are distinguished by number. The fat content was determined for basis of calculation and is also listed.

The results (Table 3) show plainly that one sample, No. 3, contained an amount of shell in excess of that consistent with good commercial practice. The figure obtained on this sample is almost three times as high as the other samples and evidently contains nearly 8.5 per cent of shell on the fat-free basis. Sample 8 was prepared by adding 0.5 gram of shell

to 6.0 grams of Sample 4. The figure of pectic acid on this sample is practically equal to that of No. 3. Moreover, the presence of a considerable amount of the darker and coarser material previously referred to was evident in this sample during centrifuging. The Referee is convinced of

TABLE 3.—*Fat and pectic acid in commercial cocoas*

NUMBER OR DESCRIPTION	FAT	PECTIC ACID (dry fat-free basis)
	per cent	*per cent*
1	21.93	0.15
2	15.49	0.18
3	15.21	0.54
4	11.99	0.18
5	12.36	0.17
6*	11.56	0.26
7	14.20	0.21
8—6 grams of No. 4 with 0.5 gram added shell	11.1	0.57
9—100% shell		4.50

* Probably contained more shell than is permitted in good factory practice.

its high shell content. The shell added to Sample 8 was that determined in No. 9. The fairly exact nature of the determination is shown by the fact that the calculated pectic acid in Sample 8 based on its ingredients is almost exactly 0.57, the figure found. The Referee believes that the method will give a close estimation of the shell content. From the results so far obtained, any sample that yields a greater figure than 0.25 per cent pectic acid is questionable. The Referee has considered making the method more sensitive by oxidizing the product obtained with chromic acid.

MILK PROTEIN IN MILK CHOCOLATE

Several analysts have complained of the length of the A.O.A.C. method for milk protein in milk chocolate and also in regard to the difficulty of the digestion, which is slow because of incessant foaming in the beginning. The Referee found that the milk protein was readily and easily dissolved by shaking the fat-free sample first with water, followed by 1 per cent sodium oxalate. The water dispersed or emulsified the milk protein, which immediately dissolved when the sodium oxalate was added. The following method appears to be both rapid and accurate:

Place 10 grams of milk chocolate in a centrifuge bottle (250 cc. or larger), and extract twice with about 100 cc. of ether by shaking until uniform, centrifuging, and decanting the supernatant ether layer each time. Place in the bottle a perforated stopper carrying a bent glass tube, and a straight glass tube that extends about one-third the way down into the bottle. Expel the ether by attaching the bent tube to the vacuum and drawing a moderate current of air through the bottle

while the latter is placed in a moderately warm (not hot) place. When the ether is expelled, measure 100 cc. of distilled water into the bottle with a bulb pipet. Stopper the bottle and shake vigorously for 3 minutes. Measure in with a pipet 100 cc. of 1% $Na_2C_2O_4$ solution. Stopper, and shake vigorously 2 minutes. Allow the bottle to stand about 10 minutes and again shake 1–2 minutes. Place the sample in the centrifuge and whirl for about 15 minutes at high speed (1800 r.p.m. if No. 1 type Sb is used).

Remove the bottle from the centrifuge and decant the supernatant liquid into a beaker. Pipet 100 cc. into a dry 250 cc. beaker and add 1 cc. of glacial acetic acid while stirring gently. Let the sample stand for a few minutes for the precipitate to partly separate and add 4 cc. of 10% tannic acid solution with stirring (solution should not be more than a week old). Allow the precipitate to separate and settle a few minutes, then filter on a Büchner funnel (7 cm. size), using moderate suction. Use as a filter a No. 589 white ribbon paper overlain with a medium layer of paper pulp prepared by shaking No. 1 Whatman filter paper with water. Transfer all the precipitate to the funnel with the aid of a policeman and a wash solution composed of 1% $Na_2C_2O_4$ to which 1 cc. of glacial acetic acid and 2 cc. of 10% tannic acid per 100 cc. have been added. Wash on the filter 1 or 2 times. Loosen the filter around the edge with a spatula. Carefully roll up and remove the filter and precipitate to a Kjeldahl flask. Transfer to the flask any particles of precipitate clinging to the funnel or spatula with small pieces of damp filter paper, add 20 cc. of H_2SO_4, 15 grams of Na_2SO_4 and 1 gram of catalyst (1 gram of Se to 5 grams of HgO). Digest after the solution clears, about $\frac{1}{2}$ to $\frac{3}{4}$ as long as the time required for the solution to become clear. (Digestion should then be complete.) Distil off the nitrogen by the usual A.O.A.C. method, except to use 50 cc. of a solution containing 300 grams of NaOH and 10 grams of $Na_2S_2O_3 \cdot 5H_2O$ in 500 cc. volume instead of the solution specified in this method. Multiply the nitrogen found by 6.38 to obtain the casein and albumin and this product by the factor 1.07 to obtain the total protein.

The factor 1.07 was arrived at after the analysis of several samples of skimmed milk powder for protein both by direct nitrogen determination and by the method given above.

When the method was applied to an authentic sample of milk chocolate of which the protein content was known, almost perfect recovery resulted. The calculated protein present was 5.60 per cent and that found by the above procedure was 5.62 per cent.

Analyses of a number of skimmed milk powders are given in Table 4.

TABLE 4.—*Protein in skimmed milk powder*

SAMPLE NO.	(1) BY DIRECT N DETERMINATION	(2) BY METHOD PRESENTED	FACTOR (COL. 1 + COL. 2)
	per cent	per cent	
1	32.74	30.69	1.065
2	32.74	30.39	1.07
3	32.85	30.41	1.08
4	35.62	33.13	1.07
5	35.62	32.95	1.08

The results in Table 4 indicate that a rather constant ratio exists between the nitrogen determined on the sample directly and that deter-

mined by the method. The Referee believes that the factor is more constant than the one now used, namely, $1.25 \times$ casein, which is obtained by precipitation with acetic acid alone.

From experiments conducted the Referee is convinced that all protein in solution is precipitated by this method. Although nitrogen is still present in the solution after the precipitation, this nitrogen appears to be due to substances other than protein, such as inorganic salts, amino acids, creatin, creatinin, uric acid, and organic bases. No protein was precipitated from this solution by boiling or by phosphotungstic acid, saturated ammonium sulfate, magnesium sulfate, or other protein precipitants. It is therefore believed that the nitrogen present is non-protein nitrogen.

LECITHIN

No work was done on lecithin because of insufficient time and the fact that no associate referee was appointed. It is hoped that work can be done on this subject next year.

RECOMMENDATIONS[1]

It is recommended—

(1) That further collaborative work be done on the pectic acid method for shell.

(2) That collaborative work be done on the method for milk protein given in this report.

(3) That work be done on the determination of lecithin in cacao products.

REPORT ON GUMS IN FOOD PRODUCTS

MAYONNAISE AND FRENCH DRESSING

By F. Leslie Hart (U. S. Food and Drug Administration, Los Angeles, Calif.), *Referee*

Edible gums are being increasingly used in the manufacture of prepared foods. While in some cases they have a legitimate use, in many cases they serve to mask adulteration. Gums have been advertised as "permitting the addition of much more water to your food product than would otherwise be possible."

The field now includes not only the true gums, such as locust bean and tragacanth, but also gel-forming substances obtained from marine algae, such as agar-agar, Irish moss, and sodium alginate. These substances are all classified chemically as polysaccharides. For convenience they will all be designated "gums," and will be included in this study. Certain other

TABLE 1.—*Collaborative results on salad dressings*

COLLABORATOR	PRODUCT	KIND AND AMOUNT OF GUM ADDED		CHARACTER OF ALCOHOL PRECIPITATE	BENEDICT	RESULTS OF QUALITATIVE SUGAR TESTS		
			per cent			MOLISCH	SELIWANOFF	TOLLEN
Doris H. Tilden U.S. Food & Drug Adm., San Francisco	Mayonnaise	Locust bean	0.2	white, opaque, partly stringy, partly granular	positive	positive	pink color	—
	French dressing	Karaya	0.2	finely flocculent, translucent	positive	positive	negative	amber
	Mayonnaise	Ghatti	0.2	flocculent, white translucent	positive sl. prec.	positive	pale pink	negative
	French dressing	Tragacanth	0.2	colorless, translucent gelatinous	positive	positive	negative	—
J. A. Kime U.S. Food & Drug Adm., Washington	Mayonnaise	Locust bean	0.15	heavy granular	positive	positive	negative	positive
	Mayonnaise	Karaya	0.15	immediate flocculent precipitate	—	positive	negative	negative
T. E. Strange U.S. Food & Drug Adm., Seattle	Mayonnaise	Ghatti	0.2	light, coherent translucent, colorless	positive	positive	pale pink	negative
	Mayonnaise	Mixture: locust bean and agar agar	0.15	white, granular, voluminous	positive	positive	positive	positive
F. Leslie Hart	Mayonnaise	Karaya	0.1	flocculent	—	—	sl. pos.	negative
	Mayonnaise	Karaya	0.2	flocculent	—	—	positive	negative
	Mayonnaise	Karaya, from diff. source	0.2	faint cloud, insufficient for further tests	negative	negative	negative	negative
	Mayonnaise	Agar-agar	0.2	blue-white jell	positive	positive	positive	positive
	Mayonnaise	Agar-agar	0.1	colorless jell	positive	positive	positive	doubtful
	French dressing	Locust bean	0.1	stringy, white	—	positive	positive	negative
	Mayonnaise	Ghatti	0.2	white, translucent	positive	positive	doubtful	positive
L. W. Ferris U.S. Food & Drug Adm., Buffalo	Mayonnaise[1]	Dextrin	0.2	granular precipitate	positive	—	negative	negative
	Mayonnaise	Locust bean	0.2	No comments	positive	positive	positive	—
	Mayonnaise	Karaya	0.15	No comments	sl. prec.	negative	negative	—

[1] Reddish-blue color with iodine.

substances, such as pectin, dextrin, and gelatin, used occasionally by food manufactuers as "thickeners," will not be considered at this time.

Methods for the detection of these gums are of interest to food chemists, particularly those engaged in regulatory activities. Due to the complexity of many prepared foods, and the varying nature of the gums themselves, it is impossible to devise one method for all products and conditions. Therefore, as methods are devised for the detection of gums in certain foods they will be reported to the Association.

Gums may be encountered in a variety of prepared foods. Among these are mayonnaise and French dressing, fruit spreads and jellies, sirups, cold-packed berries, bottlers' fruit concentrates, chocolate concentrates (powder or liquid) for dairy drinks, soft cheeses and cheese spreads, ice cream, whipping cream, catsup, prepared mustard, flavor emulsions, pudding powders, ice cream powders, and confectionery.

The work during the past year was confined to the detection of gums in mayonnaise and French dressing. A method published previously by the Referee in *This Journal*, **20**, 527, with certain modifications, was used for the work reported here. In the earlier work it was found that trichloracetic acid is the best reagent to use as a protein precipitant in separating egg or other proteins from gums. The method given herein specifies this reagent. It is the hope of the Referee that methods for the detection of gums in other food products, based on this separation, will be presented to the Association.

Relatively little work has been done on the detection of gums in food products. A bibliography on this subject, to 1937, is given in the contributed paper referred to above. Since then, a method for the detection of gums in dairy products, also specifying trichloracetic acid, was published by Racicot and Ferguson in *This Journal*, **21**, 110.

METHOD

Transfer 100 grams into a 250 cc. beaker, add 35–40 cc. of hot water, and mix thoroly. Heat to 65°–70° C. in a water bath, add 10 cc. of 50% trichloracetic acid solution in water, and maintain at 65°–70° C. until the emulsion shows signs of breaking (in no case over 10 minutes). Transfer the mixture to an 8 oz. nursing bottle, insert a pipet guard* and centrifuge 15–20 minutes at about 1200 r.p.m. (This should separate the mixture into a lower aqueous layer and an upper oily layer, with a layer of curd between. If separation does not occur, add 30–40 cc. of toluene, mix, and repeat the centrifuging.) By means of a pipet inserted through the pipet guard, remove as much of the aqueous layer as possible and filter it into a 600 cc. beaker. Add 5 volumes of alcohol and allow the mixture to stand overnight to precipitate the gums.

Decant or pipet off sufficient alcohol to leave not over 225 cc., transfer the con-

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* The device described by the writer in *This Journal*, 20, 529, 2nd par., may be used as a pipet guard. In lieu of this a piece of glass tubing, 8 mm. inside diameter and about 7½" long, may be used. The tube should be flared at the upper end, and a small cork stopper fitted onto the lower end. This stopper should be of sufficient size so that it does not rise up into the tube when the nursing bottles are centrifuged. The tube is supported in the neck of the bottle by a slotted rubber stopper. After the material in the bottle is centrifuged the cork stopper is pushed through by means of a solid glass rod and the lower layer is removed by means of a pipet inserted through the pipet guard.

tents of the beaker to an 8 oz. nursing bottle, centrifuge until the gum settles to the bottom, and decant the supernatant alcohol as completely as possible. Dissolve the residue in not over 1.5 oz. of hot water, add 1 or 2 cc. of acetic acid, and reprecipitate by adding alcohol to the 8 oz. mark on the nursing bottle. Let stand overnight, or until the precipitate becomes flocculent, centrifuge at 1200 r.p.m., and decant the alcohol. A precipitate at this point indicates gums. This may be confirmed by the following procedure.

Add 35 cc. of hot water to the precipitate in the nursing bottle, transfer to a small beaker, add 5 cc. of concentrated HCl, and boil gently for 2 minutes to hydrolyze the gums to sugars. This solution may now be used for various qualitative tests for monosaccharide sugars, as follows:

1. Transfer 1 cc. of the hydrolyzed gum solution to a test tube, neutralize with approximately $2N$ NaOH solution, using litmus paper as a reagent, remove the litmus paper, add 5 cc. of Benedict's qualitative sugar solution,† and boil vigorously 1–2 minutes. Allow to cool spontaneously. A voluminous precipitate, which may be green, yellow, or red, indicates reducing sugars.

2. *Molisch test for carbohydrates.*—Transfer 5 cc. of the hydrolyzed gum solution to a test tube, and add 2 drops of a 15% solution of alpha naphthol in alcohol. Incline the tube and slowly pour down the inner side 3–5 cc. of concentrated H_2SO_4 so that the two layers will not mix. A reddish-violet zone at the point of contact indicates carbohydrates. (A 5% solution of thymol in alcohol may be substituted for alpha naphthol.)

If sufficient solution remains, divide into two equal parts and apply the following tests:

3. *Seliwanoff test.*—Heat the hydrolyzed gum solution to boiling, and add a few milligrams of resorcinol. A red color indicates hexoses.

4. *Tollens test.*—Heat the hydrolyzed gum solution to boiling, and drop in a few crystals of phloroglucinol. A red or deep amber color indicates pentoses. Certain other sugars (as galactose) also give a positive reaction.

Samples of mayonnaise made from salad oil, egg, and vinegar, and from salad oil, egg, and lemon juice, each containing small amounts of gum, were sent to collaborators. Table 1 lists the kind and amounts of gum present in these samples, and the results submitted by the collaborators.

DISCUSSION

With the exception of Karaya all the gums used gave positive tests, in amounts as low as 0.10 per cent or 0.15 per cent. Karaya, for some reason at present unknown, gave variable results. Certain specimens of karaya gum invariably gave positive results, others either negative or doubtful. The gums used in the preparation of these mayonnaise and French dressing samples were bought from reliable importers. U.S.P. gums, acacia, agar-agar, and tragacanth complied with the criteria for these products laid down by the United States Pharmacopoeia. There is no reason to doubt the authenticity of any of the gums used.

All gums tested by the writer responded to the Benedict and Molisch

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† Dissolve 17.3 grams of Na citrate and 10 grams of anhydrous Na_2CO_3 in about 80 cc. of hot water; dissolve 1.73 grams of crystalline Cu sulfate in 10 cc. of water. Filter the alkaline citrate solution, add the Cu sulfate solution slowly, with constant stirring, and make up to 100 cc.

tests after hydrolysis. The Seliwanoff test reacted positively to karaya, agar-agar, Irish moss, and locust bean (slowly), and negatively or doubtfully to acacia, tragacanth, dextrin, sodium alginate, ghatti, and sometimes locust bean. The Tollens test gave positive reactions for karaya, tragacanth (slowly), agar-agar, locust bean, and gum ghatti. Sodium alginate turned yellow, then purple. The only gum tested showing negative or doubtful reactions to this reagent was acacia. It is noted that collaborators' reports at times show variance with these results. It is believed that this may be due to incomplete hydrolysis of the gum precipitate, or to the use of insufficient solution to respond positively.

The previous work cited showed that sugar up to 6 per cent in mayonnaise did not interfere. At that time the method was found to react positively to 0.2 per cent agar. The present investigation shows that amounts as low as 0.1 per cent gums may be detected. This is below the amount expected in commercial practice.

In the absence of starch, dextrin, or appreciable amounts of pectin, the presence of gums is indicated by an alcohol precipitate soluble in water and reprecipitated with alcohol, responding to tests for monosaccharides after hydrolysis.

The Referee has tested this method on four samples of gum-free mayonnaises, containing 8–12 per cent egg yolk, with negative results. However, time did not permit collaborative studies of gum-free mayonnaises. Samples known to be free from gums will be submitted for collaborative work next year to establish the negative application of this method.

It is recommended[1] that work on the detection of gums in mayonnaise be continued and that methods of detection of gums in other food products be studied.

REPORT ON OILS, FATS, AND WAXES

By G. S. JAMIESON (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

During the past year a collaborative study was undertaken with reference to the effect upon the results obtained for the Polenske number determination of the size of the pumice which is used to aid in the distillation of volatile acids in fats and oils. The results of this study, which has been under the direct supervision of Associate Referee R. S. McKinney, will be the subject of his report. Associate Referee Lawrence Zeleny will present a report which he and M. H. Neustadt have prepared covering the work accomplished on the refractometric determination of oil in oil seeds.

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[1]For report of Subcommittee C and action by the Association, see *This Journa'*, 22, 62 (1939).

It is recommended[1]—

(1) That changes be made in the Association's specifications for the titer thermometer, *Methods of Analysis*, A.O.A.C., 1935, 408, so as to conform to the latest specification of the National Bureau of Standards (final action).

(2) That the Kaufmann thiocyanogen method, *This Journal*, **21, 87,** be made official (final action).

(3) That the methods of the National Cottonseed Products Association for the determination of free fatty acids in crude and refined oils, *This Journal*, **21, 88,** be made official (final action).

(4) That further work be done on the refractometric method for the determination of oil in oil seeds.

(5) That further study be made of the Polenske method and the use of powdered pumice, with a view to finding a procedure for eliminating the troublesome bumping during the distillation.

(6) That the refractive index method for the determination of oil in flaxseed, *This Journal*, **22, 74,** be made official (final action).

REPORT ON REFRACTOMETRIC DETERMINATION
OF OIL IN SEEDS (SOYBEANS)

By Lawrence Zeleny (*Associate Referee*) and M. H. Neustadt
(Agricultural Marketing Service, Washington, D. C.)

In a previous report by the Associate Referee, *This Journal*, **20, 74, 421,** a refractometric method was applied to the determination of the oil content of flaxseed. Collaborative study indicates that the method is fully as accurate and reliable as the conventional petroleum-ether extraction method, and it has the advantage of being much more rapid than any previously proposed acceptable method.

The method was adopted by the Association as an official method (final action) for the determination of oil in flaxseed, and it has subsequently been used successfully as a routine procedure in a number of commercial laboratories.

The method is based upon the principle that the refractive index of the filtrate from an intimate mixture of a fat solvent and the ground flaxseed is a function of the oil content of the seed and its refractive index. Thus, if the refractive indices of both the solvent and the oil are known, the refractive index of such an extract is a measure of the oil content of the seed.

ADAPTATION OF THE REFRACTOMETRIC METHOD TO SOYBEANS

Using a mixture of halowax (α-chloronaphthalene) and α-bromonaphthalene adjusted to a refractive index of 1.63940 at 25° C. as a standard

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, **22, 63** (1939).

solvent, the writers determined the refractive indices of mixtures of this solvent in various proportions with a composite sample of freshly prepared soybean oil having a refractive index, $n_D^{25} = 1.47302$ (Table 1).

TABLE 1.—*Refractive indices at 25° C. of known mixtures of the halowax-α-bromonaphthalene solvent with a composite sample of soybean oil*

OIL IN MIXTURE	n_D^2
per cent	
0.000	1.63940
3.961	1.63031
4.898	1.62817
5.705	1.62646
6.569	1.62458
7.266	1.62297
9.154	1.61891
12.991	1.61078
16.647	1.60311
20.186	1.59612
88.879	1.48675
100.000	1.47302

The percentage of oil in the mixture obtained in the actual analysis of soybeans may be calculated from the formula:

$$\frac{100 \ Wx}{W' + Wx}$$

where W = weight of ground soybeans in grams,
       W' = weight of solvent in grams, and
       x = weight of oil in grams in 1 gram of the ground soybeans.

It may be shown with this formula that when 2 grams of ground soybeans are mixed with 5 cc. of the standard solvent, a range in soybean oil content of 10–26 per cent will correspond approximately to a range in oil content of the solvent-oil mixture of 3–7 per cent. Thus, by plotting the values shown in Table 1, the refractive index of the solvent extract corresponding to any value for oil content of soybeans may be determined. In this manner a conversion table was prepared for converting refractive index readings into soybean oil content percentages (Table 2).

Obviously this table will be strictly valid only for the analysis of soybeans whose oil has the refractive index, $n_D^{25} = 1.47302$, since an oil having that value was used in the preparation of the table. A correction table (Table 3) was therefore prepared to indicate the values to be added or subtracted from the values obtained by the conversion table for soybeans whose oils have refractive indices other than the index used as a standard. In actual practice, however, soybeans that have oils with refractive indices differing sufficiently from this standard to cause a significant error

TABLE 2.—Conversion table for determining the percentage of oil in soybeans from the refractive index of the halowax-α-bromonaphthalene extract

(1.2900 grams (5 cc.) of solvent to extract 2.00 grams of meal)

OIL per cent	n_D^{25}	OIL per cent	n_D^{25}	OIL per cent	n_D^{25}	OIL per cent	n_D^{25}	OIL per cent	n_D^{25}	OIL per cent	n_D^{25}
10.0	1.63250	12.7	1.63069	15.4	1.62895	18.1	1.62727	20.8	1.62563	.5	1.62402
.1	1.63243	.8	1.63062	.5	1.62889	.2	1.62721	.9	1.62557	.6	1.62396
.2	1.63236	.9	1.63056	.6	1.62882	.3	1.62715	21.0	1.62551	.7	1.62390
.3	1.63230	13.0	1.63049	.7	1.62876	.4	1.62709	.1	1.62545	.8	1.62384
.4	1.63223	.1	1.63043	.8	1.62870	.5	1.62703	.2	1.62539	.9	1.62378
.5	1.63216	.2	1.63036	.9	1.62863	.6	1.62696	.3	1.62533	24.0	1.62372
.6	1.63209	.3	1.63030	16.0	1.62857	.7	1.62690	.4	1.62527	.1	1.62366
.7	1.63202	.4	1.63023	.1	1.62851	.8	1.62684	.5	1.62521	.2	1.62360
.8	1.63196	.5	1.63017	.2	1.62845	.9	1.62678	.6	1.62515	.3	1.62354
.9	1.63189	.6	1.63010	.3	1.62838	19.0	1.62672	.7	1.62509	.4	1.62348
11.0	1.63182	.7	1.63004	.4	1.62832	.1	1.62666	.8	1.62503	.5	1.62343
.1	1.63175	.8	1.62997	.5	1.62826	.2	1.62660	.9	1.62497	.6	1.62337
.2	1.63169	.9	1.62991	.6	1.62820	.3	1.62654	22.0	1.62491	.7	1.62331
.3	1.63162	14.0	1.62984	.7	1.62814	.4	1.62648	.1	1.62485	.8	1.62325
.4	1.63155	.1	1.62978	.8	1.62807	.5	1.62642	.2	1.62479	.9	1.62319
.5	1.63149	.2	1.62971	.9	1.62801	.6	1.62635	.3	1.62473	25.0	1.62313
.6	1.63142	.3	1.62965	17.0	1.62795	.7	1.62629	.4	1.62467	.1	1.62307
.7	1.63135	.4	1.62958	.1	1.62789	.8	1.62623	.5	1.62462	.2	1.62301
.8	1.63128	.5	1.62952	.2	1.62783	.9	1.62617	.6	1.62456	.3	1.62296
.9	1.63122	.6	1.62946	.3	1.62776	20.0	1.62611	.7	1.62450	.4	1.62290
12.0	1.63115	.7	1.62939	.4	1.62770	.1	1.62605	.8	1.62444	.5	1.62284
.1	1.63108	.8	1.62933	.5	1.62764	.2	1.62599	.9	1.62438	.6	1.62278
.2	1.63102	.9	1.62926	.6	1.62758	.3	1.62593	23.0	1.62432	.7	1.62272
.3	1.63095	15.0	1.62920	.7	1.62752	.4	1.62587	.1	1.62426	.8	1.62267
.4	1.63089	.1	1.62914	.8	1.62745	.5	1.62581	.2	1.62420	.9	1.62261
.5	1.63082	.2	1.62907	.9	1.62739	.6	1.62575	.3	1.62414	26.0	1.62255
.6	1.63075	.3	1.62901	18.0	1.62733	.7	1.62569	.4	1.62408		

TABLE 3.—*Correction table to be applied to results from conversion table (Table 2).* [*] *Standard* $n_D^{25} = 1.4780$.
(For higher values of n_D^{25}, add correction; for lower values, subtract correction)

$n_D^{25}-1.4780$ OIL per cent	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
0.0001	.00	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01	.01
2	.01	.01	.01	.01	.01	.01	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.03
3	.01	.02	.02	.02	.02	.02	.02	.02	.03	.03	.03	.03	.03	.03	.04	.04	.04
4	.02	.02	.02	.02	.03	.03	.03	.03	.03	.04	.04	.04	.04	.05	.05	.05	.05
5	.02	.03	.03	.03	.03	.04	.04	.04	.04	.05	.05	.05	.05	.06	.06	.06	.06
6	.03	.03	.03	.04	.04	.04	.05	.05	.05	.06	.06	.06	.06	.07	.07	.07	.08
7	.03	.04	.04	.04	.05	.05	.05	.06	.06	.06	.07	.07	.08	.08	.08	.09	.09
8	.04	.04	.05	.05	.05	.06	.06	.07	.07	.07	.08	.08	.09	.09	.09	.10	.10
9	.04	.05	.05	.06	.06	.06	.07	.07	.08	.08	.09	.09	.10	.10	.11	.11	.12
10	.05	.05	.06	.06	.07	.07	.08	.08	.09	.09	.10	.10	.11	.11	.12	.12	.13
11	.05	.06	.06	.06	.07	.08	.08	.09	.10	.10	.11	.11	.12	.12	.13	.14	.14
12	.06	.06	.07	.07	.08	.09	.09	.10	.10	.11	.12	.12	.13	.14	.14	.15	.15
13	.06	.07	.07	.07	.09	.09	.10	.11	.11	.12	.13	.13	.14	.15	.15	.16	.17
14	.07	.07	.08	.08	.09	.10	.11	.12	.12	.13	.14	.14	.15	.16	.16	.17	.18
15	.07	.07	.09	.09	.10	.11	.12	.12	.13	.14	.15	.15	.16	.17	.18	.18	.19
16	.07	.08	.09	.09	.11	.12	.12	.13	.14	.15	.16	.16	.17	.18	.19	.20	.20
17	.08	.08	.09	.10	.11	.12	.13	.14	.15	.16	.17	.17	.18	.19	.20	.21	.22
18	.08	.09	.10	.10	.12	.13	.14	.15	.16	.17	.18	.18	.19	.20	.21	.22	.23
19	.09	.09	.10	.11	.13	.14	.15	.16	.17	.18	.19	.19	.20	.21	.22	.23	.24
20	.09	.10	.11	.11	.13	.14	.15	.17	.18	.19	.20	.20	.21	.23	.24	.25	.26
21	.10	.10	.11	.12	.14	.15	.16	.17	.18	.19	.20	.21	.22	.24	.25	.26	.27
22	.10	.11	.12	.13	.15	.16	.17	.18	.19	.20	.21	.22	.23	.25	.26	.27	.28
23	.11	.11	.12	.14	.15	.17	.18	.19	.20	.21	.22	.23	.24	.26	.27	.28	.29
24	.11	.12	.13	.14	.16	.17	.19	.20	.21	.22	.23	.24	.25	.27	.28	.29	.31
25	.12	.13	.14	.15	.17	.18	.19	.21	.22	.23	.24	.26	.27	.28	.29	.31	.32

[*] Per cent oil as determined from Table 2.

in the uncorrected oil content value taken from the conversion table, are seldom, if ever found.

The procedure follows:

ANALYTICAL PROCEDURE

(1) Obtain a representative sample of about 50 grams of the soybeans either by hand quartering or by use of a mechanical sampling device.

(2) Grind the beans as fine as practicable with the equipment available. The experimental roller flour mill described later is recommended for this purpose. (It is usually best to crack the beans in a coarse mill before grinding.)

(3) Weight out accurately 2 grams of the well-mixed meal and transfer the weighed sample to a 3-inch porcelain mortar that has been previously warmed to about 55° C.

(4) Add about 1.5 grams of reagent-quality sea sand and exactly 5 cc. of a halowax-α-bromonaphthalene mixture having a refractive index of $n_D^{25} = 1.63940$ ± 2. Use the utmost care in the measurement of this solution. (It is best accomplished by using an accurately calibrated pipet having a delivery time of not less than 15 seconds.)

(5) Grind the mixture in the mortar vigorously for 3 minutes, constantly scraping into the bottom the particles of meal that are thrown against the sides of the mortar.

(6) Filter the mixture into a test tube through a good quality fat-free folded filter paper of sufficiently fine porosity to yield a clear filtrate.

(7) Determine the refractive index of the filtrate at 25.0° C. to an accuracy of ± 0.00002. If the reading is made at any temperature other than 25.0° C., add 0.00043 for each degree above 25.0° C. (It is important that all temperature readings be made to the nearest 0.1°.)

(8) Using Table 2, note the percentage of oil corresponding to the refractive index reading obtained in (7). This is the uncorrected value for oil content.

(9)* In a flask shake for a minute or two about 5 grams of the meal with about 25 cc. of a good grade of low-boiling petroleum ether and filter into a small shallow evaporating dish. Carefully evaporate off the solvent on a steam bath or hot plate at low heat, and place the dish in an oven at 105° C. for 20 minutes. Cool the oil thus prepared to room temperature and determine its refractive index at 25.0° C. (The refractive index correction for temperature for soybean oil is 0.000364 per 1° C., to be added if the temperature at which the reading is taken is above 25.0°, and subtracted if below that temperature. If preferred, the sample of oil may be prepared by pressing a small sample of the ground seed in a laboratory hydraulic press and filtering the oil so obtained if it is not entirely clear.)

(10)* From the refractive index of the oil as determined in (9), subtract the value 1.4730 (refractive index at 25.0° C. of the composite sample of soybean oil used in obtaining data for the conversion table). Using this difference, determine from Table 3 the correction to be applied to the uncorrected value for oil content as determined in (8). If the difference is positive, add the correction; if negative, subtract it.

EXPERIMENTAL

As a check with which to compare results obtained by the refractometric method, the finely ground soybeans were extracted for approximately 17 hours with a petroleum ether conforming to the official spec-

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* For practical purposes Steps 9 and 10 may usually be omitted, as they add very little to the accuracy of the determination.

TABLE 4.—*Comparison of the oil content of 61 samples of soybeans as determined by the petroleum ether extraction method and by the proposed refractometric method*

SAMPLE NUMBER	DESCRIPTION OF SAMPLE	A PETROLEUM ETHER EXTRACTION METHOD (DRY-MATTER BASIS)	B REFRACTOMETRIC METHOD (DRY-MATTER BASIS)	DIFFERENCE B−A
		per cent	*per cent*	
A-5	Laredo	15.39	15.23	−0.16
B-19	Laredo, 1937; Monetta, S.C.	15.47	15.17	−0.30
B-14	Wilson—Five, 1935; Holgate, Ohio	15.99	15.99	0.00
B- 4	Haberlandt	16.20	16.20	0.00
B-21	Monetta, 1937; Monetta, S.C.	16.38	16.36	−0.02
V-49	Laredo	16.60	16.18	−0.42
V-46	Medium green	16.68	16.64	−0.04
B-34	Peking, 1936; Ohio	16.69	17.07	+0.38
A- 4	Monetta	16.76	16.39	−0.37
B-42	Avoyelles, 1937; Monetta, S.C.	17.33	17.09	−0.24
A- 6	Mammoth yellow	17.39	17.18	−0.21
C-44	Commercial	17.48	17.60	+0.12
B-41	Biloxi, 1937; Monetta, S.C.	17.64	17.47	−0.17
V-50	Otootan	17.65	17.57	−0.08
V-47	Biloxi	17.73	17.46	−0.27
B-5	Mammoth yellow	17.73	17.58	−0.15
B- 1	Virginia	17.94	18.13	+0.19
B-36	Otootan, 1937; Monetta, S.C.	18.01	18.03	+0.02
B-37	Georgian, 1937; Monetta, S.C.	18.05	18.04	−0.01
B- 3	Easycook	18.15	18.00	−0.15
B-38	Creole, 1937; Monetta, S.C.	18.19	18.19	0.00
B-20	Cayuga, 1937; Ithaca, N.Y.	18.42	18.09	−0.33
B-10	Tarheel, 1937; N.Car.	18.51	18.44	−0.07
B- 6	Tokyo	18.57	18.61	+0.04
B-11	Mandarin, 1937; Ames, Iowa	18.79	18.81	+0.02
B-29	Palmetto, 1936; Monetta, S.C.	18.80	18.38	−0.42
C-47	Commercial	18.82	18.82	0.00
B-35	Mandarin, 1936; Ohio	18.94	19.17	+0.23
B-27	White Biloxi, 1936; Monetta, S.C.	18.97	18.97	0.00
B- 8	Spooner Mandarin, 1937; Spooner, Wis.	19.03	19.27	+0.24
B-40	Charlee, 1937; Monetta, S.C.	19.06	19.12	+0.06
B- 2	Hollybrook	19.17	19.23	+0.06
V-45	Tarheel	19.17	19.39	+0.22
V-48	Virginia	19.41	19.21	−0.20
B-26	Mamloxi, 1937; Stoneville, Miss.	19.50	19.38	−0.12
B-16	Mukden, 1936; Iowa	19.70	20.00	+0.30
B-15	Mandell, 1936; Illinois	19.86	20.10	+0.24
B-28	Mamredo, 1937; Stoneville, Miss.	19.94	19.64	−0.30
A- 2	Commercial	20.12	19.95	−0.18
B-30	Hayseed, 1937; Monetta, S.C.	20.13	19.95	−0.17
C-43	Commercial	20.17	20.41	+0.24
B-25	Delsta, 1937; Stoneville, Miss.	20.21	19.90	−0.31

<div align="center">TABLE 4.—(*Continued*)</div>

SAMPLE NUMBER	DESCRIPTION OF SAMPLE	A PETROLEUM ETHER EXTRACTION METHOD (DRY-MATTER BASIS)	B REFRACTOMETRIC METHOD (DRY-MATTER BASIS)	DIFFERENCE B−A
		per cent	*per cent*	
C-48	Commercial	20.22	20.41	+0.19
B-18	Missoy, 1937; West Point, Miss.	20.24	20.47	+0.23
B- 9	Harbinsay, 1937; Urbana, Ill.	20.26	20.32	+0.06
B-13	Habaro, 1937; N.D.	20.29	20.47	+0.18
B-24	Wisconsin Early Black, 1937; Ames, Ia.	20.50	20.51	+0.01
B-23	Manchuria (13177), 1935; Holgate, Ohio	20.57	20.54	−0.03
B-17	Macoupin, 1935; Holgate, Ohio	20.64	20.94	+0.30
B-22	Scioto, 1936; Scioto, Ohio	20.79	20.99	+0.20
B- 7	Minsoy, 1937; Ames, Iowa	21.24	21.07	−0.17
B-31	Illini, 1936; Ohio	21.38	21.08	−0.30
B-39	A.K., 1936; Arlington, Va.	21.43	21.26	−0.17
A- 1	Commercial	21.51	21.22	−0.29
A- 3	Commercial	21.74	21.50	−0.24
B-32	Dunfield, 1936; Ohio	21.77	22.02	+0.25
D-53	Commercial	21.80	21.78	−0.02
E-58	Commercial	21.83	21.45	−0.38
B-12	Hudson Manchu, 1937; Vt.	21.89	21.86	−0.03
C-49	Commercial	22.07	22.08	+0.01
B-33	Manchu, 1936; Ohio	23.91	23.75	−0.16
	Average	19.16	19.12	

ifications for petroleum ether for cottonseed extraction.[1] The beans were coarsely ground and then passed three times through a roller-type experimental flour mill having two 6×6 inch rolls (40 corrugations to the inch), a speed differential between rolls of 9:7, and a speed of approximately 900 r.p.m. for the faster roll. The extraction of oil from soybeans ground in this manner is practically complete in 17 hours without the customary regrinding of the sample in a mortar. Tests were made on the extracted meal by regrinding it in a mortar with sand and extracting the reground meal for an additional 17 hours. In most cases less than 0.1 per cent of additional extract was obtained, and it appeared to consist largely of some substance other than a true oil.

Sixty-one samples of domestic soybeans were analyzed for oil content by both the petroleum ether extraction method and the refractometric method. The results of these analyses are listed in Table 4 in the order of increasing oil content. Between the two methods the average oil content for the entire series differed by only 0.04 per cent. The maximum discrepancy between the two methods was 0.42 per cent oil, and the average

[1] U. S. Dept. Agr. Service and Regulatory Announcement No. 133 (1932).

of individual discrepancies was 0.17 per cent oil. In the case of the 42 samples of this series on which duplicate determinations were made by both methods, the average difference between duplicates was 0.15 per cent oil for the extraction method, and 0.07 per cent oil for the refractometric method.

COLLABORATIVE STUDY OF THE REFRACTOMETRIC METHOD

Six samples of soybeans were analyzed by both the petroleum ether extraction method and the refractometric method by the following five collaborators.

Mayne R. Coe, Bureau of Chemistry and Soils, Washington, D. C.

R. T. Milner, U. S. Regional Soybean Industrial Products Laboratory, Urbana, Ill.

M. H. Neustadt, Agricultural Marketing Service, Washington, D. C.

W. F. Geddes, Dominion Grain Research Laboratory, Winnipeg, Canada. The Associate Referee.

These names are not listed in the order used in Table 5.

The results of this collaborative study are shown in Table 5.

TABLE 5.—*Oil content of 6 samples of soybeans as determined by 5 collaborators using the refractometric method and the petroleum ether extraction method*
(All data are averages of duplicate determinations, and are expressed as per cent oil on a dry-matter basis.)

SAMPLE NUMBER	COLLABORATOR				
	A	B	C*	D	E
Refractometric Method					
1	20.92	21.22	19.95	21.13	19.39
2	19.79	19.95	17.57	18.82	18.72
3	21.86	21.50	20.20	20.19	21.44
4	16.62	16.39	14.72	19.92	13.50
5	15.58	15.23	—	13.49	12.44
6	17.27	17.18	—	16.22	17.03
Extraction Method					
1	21.17	21.51	21.51	—	20.86
2	20.31	20.12	19.83	—	20.38
3	21.63	21.74	21.56	—	22.42
4	16.75	16.76	15.89	—	16.26
5	15.90	15.39	14.09	—	15.04
6	17.19	17.39	16.59	—	17.72

* Refractometric determinations were made with an instrument not covering the required range in refractive index at ordinary temperatures. Readings were taken at 50.75° C. and converted to 25° C., and thus the chance for error was increased appreciably.

RECOMMENDATIONS[1]

It is recommended that further work on the refractometric method be directed toward the development of a general method for determining the oil content of oil-bearing seeds and possibly other agricultural products.

Such a method conceivably could be as broad in its scope as the present extraction procedure for determining crude fat, and would be distinctly advantageous for routine work in which prompt results are required. A general method, to be of practical value, would involve the preparation of a master conversion table based upon a careful study of the relationship between the composition of solvent-oil mixtures and their refractive indices for fats and oils of different refractive index throughout the entire range encountered in nature.

No report on thiocyanogen number was given by the associate referee.

REPORT ON THE POLENSKE VALUE OF FATS AND OILS

By R. S. McKINNEY (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

In accordance with the recommendations adopted by the Association, a collaborative study was made of two methods for determining the Polenske value of fats and oils. In this study Samples A and B were the fats from blue cheese, Samples D and E were the fats from Roquefort cheese, and Sample C was 50 per cent by weight blue cheese fat and 50 per cent coconut oil. The two methods used are similar to the one described in *Methods of Analysis, A.O.A.C.*, 1935, p. 414, except that a Florence flask was specified, wash water at a temperature of 20° was used, and in Method II, 0.1 gram of powdered pumice stone was used instead of the broken pieces.

The samples were submitted to the following collaborators:

(1) I. D. Garard, New Jersey College for Women, New Brunswick, N. J.

(2) R. S. McKinney.

(3) K. R. Majors, U. S. Regional Soybean Industrial Products Laboratory, Urbana, Ill.

(4) F. H. Lehberg, Grain Research Laboratory Board of Grain Commissioners, Winnipeg, Canada.

(5) Burton Jordan, State Chemical Laboratory, Vermillion, S. Dak.

(6) M. L. Offutt, U. S. Food and Drug Administration, New York, N. Y.

(7) J. L. Perlman, State Food Laboratory, Albany, N. Y.

COMMENTS AND CONCLUSIONS OF THE ASSOCIATE REFEREE

Although this study was primarily on the Polenske value determination, it was thought worth while to include also the Reichert-Meissl value

TABLE 1.—*Reichert-Meissl values*

COLLABORATOR	METHOD I (PIECES OF PUMICE)	av.	METHOD II (FINE PUMICE)	av.
	Sample A			
1	26.15; 25.02	25.6	25.52; 24.68	25.1
2	25.3 ; 25.1	25.2	25.6 ; 25.7 ; 25.8	25.7
3	27.1 ; 27.0 ; 27.1	27.1	26.6 ; 26.3	26.5
4		26.2		26.31
5	25.6 ; 25.7 ; 25.9	25.7	25.7 ; 26.1 ; 26.2	25.6
6	25.9	25.9		25.6
7	25.9 ; 26.5	26.2	26.7 ; 26.2	26.5
	Av.	26.3		25.9
	Av. variation	0.5		0.4
	Sample B			
1	23.12; 22.73	23.0	22.97; 23.2	23.1
2	22.7 ; 22.5	22.6	22.0 ; 22.3 ; 22.3 ; 22.9	22.4
3	22.6 ; 22.8	22.7	23.2 ; 23.4	23.3
4		23.77		23.4
5	22.83; 23.10; 23.21; 23.07; 23.43; 22.88	23.1	23.02; 22.91; 23.05	23.0
6		22.6		23.2
7	21.9 ; 22.5	22.2	22.9 ; 22.1	22.5
	Av.	22.9		23.0
	Av. Variation	0.4		0.3
	Sample C			
1	10.50; 18.83	10.7	10.65; 10.58	10.62
2	10.5 ; 10.6 ; 10.7	10.6	10.6 ; 10.8	10.7
3	10.8 ; 10.9	10.85	11.0 ; 11.0 ; 11.2	11.1
4		10.83		11.09
5	11.63; 11.30; 11.63; 11.52; 11.77	11.56	11.58; 11.52; 11.52; 11.58	11.55
6		11.1		11.2
7	10.6 ; 10.7	10.7	10.7 ; 11.1	10.9
	Av.	11.0		11.0
	Av. Variation	0.4		0.3
	Sample D			
1	27.22; 26.65	26.9	27.46; 27.52	27.5
2	28.1 ; 28.3 ; 28.5	28.3	27.4 ; 27.4	27.4
3	29.0 ; 29.0	29.0	28.8 ; 29.3 ; 29.4	29.2
4		30.14		30.21
5	29.14; 29.21; 29.45	29.3	29.38; 29.28; 29.13; 29.61	30.4

TABLE 1.—*Reichert-Meissl values—(Continued)*

COLLABORATOR	METHOD I (PIECES OF PUMICE)		METHOD II (FINE PUMICE)	
		cc.		*cc.*
6		28.2		28.4
7	29.3 ; 29.6	29.5	28.9 ; 29.3	29.1
	Av.	28.8		29.9
	Av. Variation	0.8		0.9
	Sample E			
		cc.		*cc.*
1	29.82; 29.65	29.7	29.61; 30.44	30.0
2	29.1 ; 29.3 ; 29.8	29.4	28.9 ; 29.1	29.0
3	30.3 ; 29.3 ; 29.8 ; 29.6	29.8	29.6 ; 30.2	29.9
4		30.51		30.18
5	30.82; 30.82; 30.76	30.8	30.26; 30.71; 30.71; 30.54	30.7
6		29.9		30.0
7	30.1 ; 30.2	30.2	29.8 ; 30.0	29.9
	Av.	30.0		30.0
	Av. Variation	0.4		0.3

TABLE 2.—*Polenske values*

COLLABORATOR	METHOD I (PIECES OF PUMICE)		METHOD II (FINE PUMICE)	
	Sample A			
		cc.		*cc.*
1	1.88; 1.75	1.82	2.42; 2.47	2.45
2	1.37; 1.39	1.38	1.65; 1.78; 1.92	1.79
3	2.1 ; 2.0 ; 1.9	2.0	2.1 ; 2.4	2.25
4		2.45		2.56
5	1.58; 1.48; 1.75	1.60	1.92; 1.94; 1.96	1.94
6		1.63		1.80
7	2.1 ; 2.1	2.1	2.2 ; 2.4	2.3
	Av.	1.85		2.16
	Av. Variation	0.28		0.25
	Sample B			
		cc.		*cc.*
1	2.12; 1.93	2.03	2.73; 2.84	2.79
2	1.89; 1.91	1.90	1.80; 1.92; 2.22	1.98
3	1.90; 2.0 ; 2.1	2.0	1.8 ; 1.8	1.80
4		2.45		2.42
5	1.82; 1.95; 1.85; 1.80	1.86	2.12; 2.82	2.47
6		2.03		2.03
7	2.4 ; 2.2	2.3	2.8 ; 2.5	2.65
	Av.	2.05		2.30
	Av. Variation	0.16		0.31

TABLE 2.—*Polenske values*—(*Continued*)

COLLABORATOR	METHOD I (PIECES OF PUMICE)	av.	METHOD II (FINE PUMICE)	av.
		Sample C		
		av.		av.
1	7.13; 7.21	7.17	8.26; 8.76	8.51
2	6.22; 6.24	6.23	7.21; 7.44	7.33
3	5.1 ; 5.6	5.35	7.1 ; 7.5 ; 7.4	7.3
4		7.7		7.7
5	7.50; 5.66; 5.85; 6.42; 5.83; 6.80	6.34	6.95; 7.30; 7.75; 7.10	7.23
6		5.76		6.12
7	7.2 ; 8.4	7.8	7.1 ; 8.2	7.65
	Av.	6.62		7.41
	Av. Variation	0.80		0.47
		Sample D		
		av.		av.
1	3.03; 3.36	3.20	4.42; 4.51	4.47
2	2.45; 3.12; 3.75	3.12	3.70; 3.71	3.71
3	3.7 ; 3.2	3.45	3.6 ; 3.9 ; 4.6	4.40
4		5.08		5.13
5	3.40; 3.45; 3.25	3.67	4.10; 4.55; 2.65; 4.20	3.88
6		4.04		4.75
7	3.6 ; 3.4	3.5	4.6 ; 4.8	4.7
	Av.	3.72		4.43
	Av. Variation	0.48		0.39
		Sample E		
		av.		av.
1	4.32; 6.39	5.36	5.15; 4.49	4.82
2	3.64; 3.64; 3.72	3.67	4.52; 4.54	4.53
3	5.4 ; 4.7 ; 4.0 ; 4.6	4.7	5.0 ; 5.0	5.0
4		4.23		5.10
5	4.55; 4.35; 4.55	4.48	3.2 ; 5.1	4.15
6		4.10		4.67
7	4.0 ; 4.1	4.05	5.2 ; 5.3	5.25
	Av.	4.37		4.79
	Av. Variation	0.41		0.29

results. These same results are being used by I. D. Garard, Associate Referee on Cheese and also on the Difference between Dairy Products from Cow's Milk and those from Milk of other Animals. Garard prepared the samples and sent them to the collaborators for study.

In regard to the Reichert-Meissl value determination, it was found that the same results were obtained with both methods, although slightly better agreement was obtained when the fine pumice was used.

In regard to the Polenske value determination, it was found that

Method II, with the fine pumice, gave results about 0.50 per cent higher than did Method I, with the pieces of pumice. The former method gave results showing a slightly better agreement between individual analysts, 0.34 per cent instead of 0.43 per cent average variation. However, any better agreement was overshadowed by the fact that more than half of the collaborators had considerable trouble with bumping.

It is therefore recommended[1] that further study be made of the Polenske method, with powdered pumice, with a view to finding a procedure for eliminating the troublesome bumping during the distillation.

REPORT ON MICROCHEMICAL METHODS
ALKOXYL DETERMINATION
By E. P. CLARK (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.), *Referee*

The past year has been the first period since the appointment of the Referee on Microchemical Methods that interest in or cooperation on the subject has been secured. Advantage of this fact was immediately taken, and as a start the methoxyl and ethoxyl method previously reported by the Referee, *This Journal*, 15, 136, was submitted to collaborative study. There were several reasons for this: First, the method has been recommended for adoption; second, it has been used by Milstead, *Ibid.*, 21, 543, for estimation of the purity of guaiacol and its derivatives; and third, the method is precise and has been largely adopted in research and industrial laboratories.

Samples of two preparations, lignin and rotenone, and directions for conducting the determination were submitted to a number of analysts, nine of whom reported. Their findings are given in Table 1.

From these results the following conclusions are drawn:

The variations in the checks on lignin can be accounted for, to some extent at least, by the fact that its moisture content varies with the relative humidity, and consequently the different times and places at which the samples are weighed influence the values obtained unless special precautions are taken. In the case of the samples submitted no special instructions of this type were given. With rotenone the moisture factor did not enter, and the values obtained are consequently more consistent.

The outstanding result of the study, however, is that most collaborators obtained close agreements in their check analyses and the average results of all analyses are very near the true values of the compounds submitted.

Aside from this study, the Referee's experience in analyzing over 500 pure compounds, together with private reports from other laboratories in which at least several thousand determinations have been made, indicates that the variations in relative results are not due inherently to the

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[1] For report of Subcommittee C and action by the Association, see *This Journal*, 22, 63 (1939).

method, but rather to the balances, methods of weighing, and the stand-
ardization of the volumetric solutions employed. It is therefore suggested
that the method be adopted as official.

The procedure recommended is, with the exception of a few minor
changes, the same as previously published. A change in the apparatus is

TABLE 1.—*Collaborative results on determination of methoxyl in lignin and rotenone*

ANALYST	LIGNIN		ROTENONE	
	OCH₃ FOUND	DIFFERENCE	OCH₃ FOUND	DIFFERENCE
	per cent	*per cent*	*per cent*	*per cent*
1	12.81	0.03	15.71	0.04
	12.78		15.67	
2	12.90	0.18	15.90	0.03
	12.72		15.93	
3	12.50	0	15.6	0
	12.50		15.6	
4	12.91	0.16	15.78	0.04
	12.75		15.74	
5	15.61	0.04	15.73	0.03
	12.65		15.70	
6	12.83	0.10	15.83	0.14
	12.93		15.97	
7	12.73	0	15.71	0.04
	12.73		15.67	
8	12.62	0.20	15.37	0.04
	12.42		15.41	
9	12.34	0.04	15.37	0.09
	12.38		15.45	
Average	12.67	0.08	15.68	0.05
Calculated	12.79		15.7	

made with the hope that apparatus manufacturers will follow the speci-
fications of the more simply fabricated design more closely than they did
with the previous apparatus. Furthermore, the new design can readily
be adapted to the determination of high alkoxyls by slipping a water
jacket over the air condenser and scrubber and heating the entire appara-
tus to the necessary temperature. The use of less potassium acetate-

acetic acid solution than formerly used is recommended, as it insures a clearer and sharper end point.

This method was published in *This Journal*, 22, 100.

REPORT ON MICROBIOLOGICAL METHODS

By ALBERT C. HUNTER (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The program planned for the development of microbiological methods is progressing, even though slowly. Those who have followed the program in this field will recall that at the beginning some time was devoted to a study of fundamental procedures and matters of technic involved in the bacteriological examination of canned foods, following which attention was turned toward the development of proposed methods for the microbiological examination of specific classes of products. Methods have now been set up for the bacteriological examination of canned meats, canned fish, canned vegetables, canned tomato products, and sugar. These methods are now being subjected to collaborative study. Inasmuch as these studies have not yet been carried far enough to provide bases for reports, there are no statements to be made this year by the Associate Referees in charge of those methods which have been presented heretofore, and have been published.

Last year in expansion of the program, the appointment of an associate referee on microbiological methods for the examination of eggs and egg products was recommended, and as a result, at this meeting a proposed method for the bacteriological examination of frozen eggs is being presented.

While it is believed that the methods for canned meats and for sugar have probably been studied sufficiently to warrant a recommendation for their adoption as tentative methods, it appears to be the better policy to withhold such recommendation until further collaborative work on the closely related methods for canned foods other than meats can be completed. The program then for the ensuing year calls for extensive collaborative work on the methods now set up without expanding the field further at this time.

It is recommended that the present Associate Referees on Canned Vegetables, Canned Tomato Products, Canned Fish Products, Canned Meats, Sugar, and Eggs and Egg Products be reappointed.

No reports were given on the following subjects relating to canned products: Fish, meats, vegetables, tomato products, and sugar.

REPORT ON MICROBIOLOGICAL METHODS FOR EXAMINATION OF FROZEN EGG PRODUCTS

By Roy Schneiter (Bacteriological Section, U. S. Food and Drug Administration, Washington, D. C.),

Associate Referee

Egg products may be classified as follows:

1. Shell eggs
2. Broken out eggs (liquid)·
    (a) Whole eggs
    (b) Egg whites
    (c) Egg yolks
3. Dried eggs:
    (a) Albumin or whites
    (b) Yolk
    (c) Whole or mixed eggs
4. Frozen eggs:
    (a) Albumin or whites
    (b) Plain yolk
    (c) Sugar yolk
    (d) Salt yolk
    (e) Glycerine yolk
    (f) Whole or mixed eggs.

The bacteriological examination of egg products involves different factors for each different class, but only methods for the microbiological examination of frozen egg products are to be considered at this time.

The major portion of the output of the frozen egg industry is utilized in the preparation of food products that receive little or no heat processing during their manufacture. It is, therefore, essential that the frozen egg products used be free from large numbers and types of viable microorganisms, which may induce rapid spoilage or be of potential danger to health when incorporated in foods.

Microbiological methods for the examination of frozen egg products should include procedures for the determination of (1) the total numbers of microorganisms and (2) the incidence of types of microorganisms that may be dangerous to health or conducive to food spoilage.

While the presence of large numbers of viable microorganisms in frozen egg products is indicative of the use of poor quality shelf stock, unsatisfactory manufacturing procedures, or insanitary plant conditions, a high incidence of coliform organisms and hemolytic types of bacteria may be, in addition, indicative of potential danger to health.

The following microbiological methods are proposed:

I. SAMPLING

EQUIPMENT

(1) Electric drill with auger (12"×1"), (2) alcohol burner, (3) alcohol (95% C_2H_5OH), (4) absorbent cotton, (5) two tablespoons, (6) sample containers (sterile

1 qt. or 1 pt. Mason jars), (7) hammer and steel strip (12"×2"×0.25") or other tool for opening cans, (8) water pail, (9) towels, and (10) record book, pencils, etc.

PROCEDURE

Select a representative number of cans from lot (square root of total). Note and record all marks of identification, for example: firm name and location, brand, type product, code or lot numbers, etc. Sterilize auger and tablespoons by sponging off with alcohol-soaked cotton and heating in flame of alcohol burner. Wash drill and spoons in pail of water and re-sterilize after each container sampled. Open the containers aseptically. Drill three cores equidistant between side and center of can and one-third of periphery apart. Transfer drillings from can to sample container with sterile tablespoon. Examine product organoleptically by smelling at opening of drill-hole after sample is removed. (Heat produced by electric drill intensifies odor of egg material, thus facilitating the organoleptic examination.) Record odors as normal, putrid, sour, or musty.

Refrigerate samples with dry ice if analysis is to be delayed or sampling point is at some distance from laboratory. Carry out sampling procedure under as nearly aseptic conditions as possible.

II. ANALYTICAL PROCEDURE

PREPARATION OF SAMPLE

Thaw frozen egg material as rapidly as possible in order to prevent an increase in the numbers of microorganisms present and at temperatures sufficiently low to prevent destruction of microorganisms (20°–30° C.). (Frequent shaking aids in thawing the frozen material. Thawing temperatures may be maintained by the use of a water bath.)

Thoroughly mix each thawed sample with spoon or electric stirrer before analysis. Prepare a 1–10 dilution by aseptically weighing 5.0 grams of egg material into a wide-mouthed glass-stoppered bottle containing 45 grams of sterile physiological salt solution (0.85% NaCl per 1,000 cc. distilled water) and 1 tablespoonful of glass shot. Agitate the 1–10 dilution thoroughly to insure complete solution or distribution of the egg material in the diluent. Prepare serial dilutions from 1–100 to 1–100,000,000 for inoculation into various culture media. Inoculate all media within 15 minutes after the sample is prepared.

PLATE COUNTS

Inoculate duplicate plates with 1 cc. portions of all dilutions from 1–1,000 to 1–1,000,000. (Nutrient agar or dextrose agar may be employed as plating media.) Incubate one set of plates at 20°–30° C. for 3 days and the second set of plates at 37° C. for 3 days. Express final results as numbers of viable microorganisms per gram of egg material. (Uniformly higher counts are always obtained on plates incubated at 20°–30° C.)

INCIDENCE OF COLIFORM GROUP

Inoculate 1.0 cc. portions from suitable dilutions (1–10 to 1–100,000,000) of egg material into fermentation tubes of lactose broth. Incubate at 37° C. for 24–48 hours.

Streak Levine's eosin methylene blue agar plates from all lactose broth cultures showing gas production. Incubate plates at 37° C. for 24–48 hours. Examine E. M. B. agar plates for colonies of microorganisms of the coliform group.

Inoculate from colonies of the coliform types of bacteria appearing on E. M. B. agar plates to nutrient agar slants. Incubate at 37° C. for 24 hours. Purify cultures for further study.

Obtain biochemical reactions of purified cultures by Kovac's test, indol production; methyl red (M. R.) and Voges Proskauer (V. P.) tests; and Koser's sodium citrate test, utilization of sodium citrate as the sole source of carbon.

NOTE: Follow procedure recommended in *Standard Methods of Water Analysis*, 8th Ed., 1936, of The American Public Health Association for Biochemical Reactions.

HEMOLYTIC TYPES OF MICROORGANISMS

Inoculate petri plates with 1 cc. portions of all dilutions from 1–100 to 1–1,000,000. Pour plates with veal-infusion agar containing 6% of defibrinated horse, sheep, or rabbit blood (0.6 cc. of blood per 10 cc. of media). Cool agar to 40° C. and add blood just prior to pouring plates. Incubate plates for 24 hours at 37° C. Express final results as numbers per gram.

ANAEROBIC TYPES OF MICROORGANISMS

Inoculate tubes containing chopped meat media with 1 cc. portions of all dilutions from 1–10 to 1–100,000. Incubate for 3 days at 37° C.

DIRECT MICROSCOPIC COUNTS

Place 0.01 cc. of the 1–10 or 1–100 dilutions of egg material on a clean microsopic slide and spread over an area of 1.0 sq. cm. Permit the smear preparation to dry on a level surface at 30°–40° C. Proceed as directed in *Standard Methods of Milk Analysis* of the American Public Health Association (latest edition). Multiply total count by 10 or 100, since the original smear preparation was made from a 1–10 or 1–100 dilution in order to obtain the numbers of bacteria per gram of egg material.

III. CULTURE MEDIA

STANDARD METHODS MEDIA

Prepare as recommended in *Standard Methods of Water Analysis*, 8th Ed., 1936, of The American Public Health Association.

Nutrient agar, lactose broth, Levine's eosin methylene blue agar, tryptophane broth, methyl red-Voges Proeskauer peptone medium, and Koser's sodium citrate medium.

Other media include (a) *Dextrose agar.*—Standard nutrient agar plus 1.0% dextrose. (b) *Veal infusion agar.*—Ground lean veal, 500.0 grams, and distilled water, 1000.0 cc. Infuse overnight in refrigerator and strain through cheesecloth without pressure. Make up to original volume and skim off any fat. Steam in Arnold 30 minutes and filter through paper. Add:

	per cent		grams
Peptone (Difco)	1.0	or	10.0
NaCl	0.5	or	5.0
Agar	1.5	or	15.0

Steam in Arnold to dissolve. Adjust reaction to *p*H 7.6 and steam in Arnold 15 minutes. Filter through Büchner funnel with paper pulp mat, by the aid of suction. Use egg albumin for clarification when necessary. Distribute 10 cc. quantities into test tubes or 80 cc. quantities into bottles. Sterilize at 15 lbs. pressure for 20 minutes. Final *p*H 7.4. (For hemolytic tests agar should be cooled to 40° C. and 6% of defibrinated horse, sheep, or rabbit blood added prior to pouring plates (0.6 cc. of blood per 10 cc. of media.)

(c) *Holman's cooked meat medium (alkaline).*—Distilled water, 1000 cc., ground fresh lean beef, 500 grams, bacto-peptone, 5 grams, and C. P. NaCl, 5 grams. Infuse the beef-water mixture overnight in refrigerator. Strain through several layers of cheesecloth and press out broth, retaining the meat press cake. Add distilled

water to the infusion to make 1 liter. Add the peptone and heat in the Arnold or boil 10 minutes. Filter, and add salt. Add normal NaOH until alkaline to phenolphthalein. Heat in Arnold 15 minutes to clear and filter. Distribute the pressed-out beef remaining from the infusion into medium sized tubes (150×20 mm.), approximately 2 grams into each tube, and add 10 cc. of the cleared alkaline broth. Sterilize in the autoclave at 15 lbs. pressure for 15 minutes. Final reaction should be pH 7.2–7.4. Store in refrigerator. Prior to using, boil the tubed medium for at least 10 minutes to expel adsorbed oxygen and cool promptly in a water bath.

The Manual Methods for Pure Culture Study of Bacteria of the Society of American Bacteriologists should be used as a guide for the further study of microorganisms obtained in the cultural procedures described.

No report on feeding stuffs was given by the associate referee.

REPORT ON SAMPLING FEEDING STUFFS

By L. M. JEFFERS (Bureau of Field Crops, California Department of Agriculture, Sacramento, Calif.), *Associate Referee*

In connection with regular feeding work, the Associate Referee carried on limited tests for the purpose of determining the efficiency of different types of samplers and different methods of sampling. In these tests the inspector was instructed to attempt to get a representative sample under each method. The analytical results obtained in these comparative methods of sampling are surprisingly close, but data obtained are not sufficiently extensive to warrant introduction at this time.

There may be certain limits in the setting up of definite sampling instructions, and the attempt of the inspector to secure truly representative samples is most important.

No information has been secured from other agencies relative to sampling methods and suggestions for definite instructions. The gathering of such information is desirable.

It is recommended,[1] therefore, that the study be continued with the hope that there may be developed standard methods of sampling feeding stuffs.

REPORT ON ASH IN FEEDING STUFFS

By J. L. ST. JOHN (Division of Chemistry, Agricultural Experiment Station, Pullman, Wash.), *Associate Referee*

It has been observed that difficulty is experienced in determining the ash of mixed feeds that contain added calcium carbonate in the form of oyster shell or limestone. Results obtained by different laboratories have

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[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 50 (1939).

been found to be irregular. The Association therefore appointed a referee to study methods of determining ash in feeds. The work this year was confined to feeds to which calcium carbonate had been added.

The major part of the work done on the determination of ash during recent years appears to relate to methods useful for the determination of ash in flour, and most of the papers were published in *Cereal Chemistry*. L. H. Bailey (1937) summarized the work on methods of ashing cereal products, much of which has been confined to a study of flour. He made a further study of the application of certain of these methods to the determination of flour ash and also to a limited extent the ashing of bran and low-grade flour. He concluded that the most satisfactory method was a modification of the magnesium-acetate method where, with bran, a two gram sample was used, three times the amount of magnesium-acetate solution was added, and ashing was continued for 1.5 hours. He states that the results duplicated those obtained by the official A.O.A.C. method. Six grams of anhydrous magnesium-acetate per liter of alcohol was used; 9 cc. of this solution was used with bran. The temperature of the muffle furnace was 700° C.

The magnesium-acetate method and other modified methods of ashing are described in *Cereal Laboratory Methods*, and in *This Journal*, 20, 69. It was recommended by this Association that this method be adopted as official (first action), *Ibid.*, 21, 389, for the determination of ash in flour, macaroni products, and baked products.

Blasdale, in his text on quantitative analysis, gives the following table for the dissociation pressure of calcium carbonate:

°C.	mm.
20—	2.2×10^{20}
200—	7.8×10^{9}
400—	0.3×10^{3}
500—	0.15
600—	2.98
700—	31.2
800—	208
882—	760
900—	984

He also states that "although precipitated calcium carbonate rapidly attains true equilibrium when heated, this is not true of naturally occurring samples. The latter can be heated to temperatures in excess of that named for several hours before appreciable decomposition occurs."

Willard and Furman, in their text on quantitative analysis, make the following statements in discussing the determination of calcium:

$$CaCO_3 \text{ (heated to } 850° \text{ C.)} = CaO + CO_2.$$

This reaction is rapid at 850°, but begins at about 600°. At 550° the carbonate loses some carbon dioxide. The dissociation of the carbonate is a true equilibrium reaction. At 500° the dissociation pressure of calcium carbonate is 0.11 mm., a

value less than the partial pressure of carbon dioxide in ordinary air. Consequently, no dissociation of the carbonate will occur at this temperature. At 600° the dissociation pressure of calcium carbonate is 2.35 mm., and therefore carbon dioxide will slowly be lost. At 890° the dissociation pressure is 690 mm. A method of determining calcium as calcium carbonate by heating the oxalate to about 700° C. in an atmosphere of carbon dioxide has been described. Since calcium oxide is not reduced by carbon, no special care is necessary in burning off the filter. Care must be taken, however, to maintain a very high temperature during ignition and to heat the material sufficiently long to decompose all calcium carbonate. The dissociation is assisted by the removal of the carbon dioxide evolved, and it can be completed below 800° C. if the calcium carbonate is heated in a current of air free from carbon dioxide.

The A.O.A.C. method for feeds specifies that the material shall be burned at a low red heat not exceeding dull redness until free from carbon, but does not define these statements. The A.A.C.C. method specifies ignition at approximately 550° C. (dull red) until a light gray ash results, or until no further loss in weight occurs. The term "dull red heat" is indefinite. The ignition of a sample until it ceases to lose weight is impractical for many laboratories. Also, there is a question of the meaning of "free from carbon" when calcium carbonate is included in the sample. It is believed that the need for standardization of the ash method is evident, particularly in view of the results presented in this paper.

To collect further information regarding the results obtained by using different conditions in ashing the same material, the Associate Referee sent three samples to forty collaborators throughout the country, and after correspondence with them regarding the method to be used in the study this year, furnished the following method for this survey:

Weigh 2 gram samples of feed in porcelain crucibles, place in a cold muffle furnace, bring rapidly to the desired temperature, and continue at that temperature for the specified time.

The collaborators were requested to make duplicate determinations and report to the second decimal place. Each of the three samples was to be ashed at 550, 600, 650, and 700° C. for 2, 5, and 16 hours after the furnace reached temperature. It was specified that the temperature should be accurately controlled and measured with a pyrometer. Somewhat detailed information was requested regarding the kind of muffle used, the type of crucible, the accuracy with which it was possible to control the temperature, and other observations.

The three samples of feed submitted to the collaborators were based on a poultry and a dairy mixed ration recommended by the departments of the State College of Washington that work along these lines. The poultry ration was a chick starting mash with the following ingredients:

per cent
40 Ground yellow corn
10 Ground wheat

   12 Finely ground heavy oats
   15 Wheat bran
    5 Dehydrated alfalfa
    5 Meat scrap
    5 Fish meal
    5 Skim milk powder
    2 Ground oyster shell or limestone
    1 Salt
    1 Biologically tested cod-liver oil or fish oil or its equivalent in
      concentrated Vitamin D

This ration, mixed by the poultry department, constituted Sample 1.
It will be noted that it contains 2 per cent of calcium carbonate as ground
oyster shell. Sample 2 was composed of an additional portion of this same
ration to which a further 4 per cent of precipitated calcium carbonate
had been added. Sample 3 was the dairy ration, composed of one-third
peas, one-third barley, one-third oats, plus 1 per cent bone meal and 1

TABLE 1.—*Results on screened samples*

SAMPLE NO.	1	2	3
	per cent	*per cent*	*per cent*
20 mesh	2.7	4.7	7.1
40 mesh	30.0	36.7	49.3
60 mesh	20.9	17.0	16.2
80 mesh	26.3	17.8	12.5
100 mesh	8.7	5.0	3.1
Less than 100 mesh	10.1	17.3	9.8
	98.7	98.5	98.0

per cent sodium chloride. To this basic ration was added 10 per cent of
precipitated calcium carbonate. The ration represented by Sample 1
ordinarily runs 6.5 per cent of ash, and that represented by Sample 3,
without the calcium carbonate, ordinarily runs around 5.5 per cent of
ash. If completely decomposed to calcium oxide during ashing the calcium
carbonate incorporated in Sample 2 would add 2.24 per cent ash to the
sample, while in Sample 3, 5.6 per cent ash would be added.

The poultry ration used in Samples 1 and 2 was very thoroughly
ground through a Wiley mill with the coarse screen. It was then thor-
oughly mixed on a cloth and divided into two portions; the 4 per cent
calcium carbonate was added to one portion, which was again very thor-
oughly mixed on a mixing cloth. Particular attention was given to the
mixing of all samples. The dairy ration was ground through the Wiley
mill, mixed, and then mixed with the calcium carbonate. Samples were
placed in 4 ounce bottles with screw tops and shipped to the collaborators.

Recently weighed portions of all three samples were put through a
screen. The results are given in Table 1.

TABLE 2.—Collaborative results

	COLLABORATOR NO.	550—2 HRS.			550—5 HRS.			550—16 HRS.			600—2 HRS.		
1. Adams	22	8.34	11.15	9.94	8.38	10.16	8.95	9.21	11.86	9.63	7.91	10.02	7.96
2. Becknell	30	9.58	12.30	11.41	9.28	12.21	11.11	8.20	10.00	8.94	9.00	11.83	10.50
3. Brooke	2	9.61	12.34	11.50	8.72	11.54	10.55	9.11	11.22	8.06	8.90	11.84	10.60
4. Clulow	33	9.24	11.86	11.34	8.35	11.93	8.31	8.26	9.76	7.95	8.46	10.00	7.82
5. Geagley	9	9.92	12.57	11.82	8.77	11.55	10.53	9.88	12.51	11.71	8.39	10.84	9.54
6. Grattan	10				9.88	12.53	11.72				9.71	12.50	11.45
7. Halvorsen	12							9.23	12.51	11.41	9.32	12.30	11.22
8. Hand	13	9.61	12.86	11.04	9.30	12.05	10.85	9.23	12.08	10.30	8.50	11.58	9.49
9. Haskins	15	9.60	11.98	11.17	8.43	10.12	8.67	8.48	10.13	7.98	8.19	10.08	7.92
10. King	17	8.75	11.85	10.15	8.52	11.07	9.43	7.82	10.07	7.77	7.73	11.24	
13. Montzheimer	34	8.27	10.84	9.56	8.30	10.11	8.06	8.33	10.16	8.16	8.21	9.81	8.18
14. Nixon	6	9.28	12.27	10.05	9.21	12.04	11.11	8.74	11.20	9.30	9.25	12.10	11.02
15. Randall	29	9.40	12.44	10.76	9.40	12.34	10.63	9.29	12.16	10.65	8.94	11.95	10.88
16. Struve	23	9.60	12.64	11.26	9.52	12.50	10.44	8.78	11.43	9.31	9.57	12.45	11.17
17. Tobey	24	9.84	12.36	11.22	8.40	10.78	9.67	8.33	10.19	8.01	8.76	11.78	9.78
18. Walker	25	9.54	12.93	11.35	9.48	12.44	11.18						
19. Ziegler	27	9.21	12.14	10.96	8.90	11.54	10.01	8.15	10.21	9.33	8.34	9.94	8.26
20. Walls											8.10	12.35	10.80
Averages		9.34	12.21	10.93	8.96	11.63	10.16	8.78	11.14	9.38	8.66	11.33	9.79

TABLE 2.—Collaborative results—(Continued)

	600—5 HRS.			600—10 HRS.			650—2 HRS.			650—5 HRS.			650—16 HRS.		
1.	8.16	10.04	8.04				8.21	9.76	7.85	7.85	9.85	7.62	7.93	9.73	7.47
2.	8.27	11.41	8.19	8.22	9.87	7.88	8.26	10.01	8.16	8.12	9.99	8.01	7.55	9.33	7.65
3.	8.45	9.96	7.90	7.54	9.81	8.30	8.95	11.23	9.40	8.26	9.69	7.62	7.17	8.65	7.43
4.	8.20	10.03	7.83	7.75	9.40	7.32	7.81	10.04	7.82	7.30	9.43	7.62	7.73	9.25	7.51
5.	8.33	9.91	7.89	8.05	9.62	7.60	8.35	9.81	7.72	8.08	9.78	7.61	8.43	10.27	8.48
6.	9.26	12.13	10.93	8.50	11.11	9.69	8.67	11.60	10.53	8.41	10.45	8.58	8.23	10.12	7.78
7.	8.34	9.67	7.51	8.72	11.16	9.99	8.70	11.56	10.36	7.82	9.80	7.47	7.99	9.70	7.60
8.	8.29	10.02	7.91	7.88	9.94	7.71	8.51	10.37	7.79	8.14	10.04	7.77	7.44	9.69	7.38
9.	8.04	9.93	7.77	8.27	10.03	7.93	8.43	10.18	8.19	7.98	9.59	7.87	7.54	10.03	7.91
10.	7.87	10.43		7.99	10.84		7.93	10.27	7.17	7.82	10.22	8.08	7.33	8.88	7.60
13.	8.15	9.80	8.04	7.85	9.49	7.78	7.86	9.78	7.73	7.60	9.51	7.69	7.39	9.43	7.63
14.	8.66	12.02	11.03	8.01	9.91	7.93	8.97	11.79	10.83	8.08	10.06	7.75	8.01	9.93	7.91
15.	8.87	11.88	10.45	8.60	11.18	10.10	8.57	10.70	9.51	8.16	10.06	8.06	8.45	10.25	8.16
16.	9.23	11.71	10.47	9.27	11.40	8.38	9.40	12.22	10.92	8.35	10.56	8.34	6.65	8.36	7.17
17.	8.05	9.89	7.81	8.08	10.04	7.99	8.57	10.02	8.01	7.83	9.60	7.61	7.75	9.77	7.84
18.							8.50	10.16	9.25	8.29	10.09	8.59	7.59	9.50	7.77
19.	8.36	9.83	7.93	8.08	9.88	7.85	8.08	9.75	8.04	7.72	9.71	7.53			
20.	8.30	11.15	9.38				8.01	10.07	8.02	8.00	10.37	8.00			
Av.	8.45	10.67	8.84	8.23	10.34	8.50	8.43	10.52	8.74	7.99	9.93	7.88	7.72	9.58	7.73

TABLE 2.—Collaborative results—(Continued)

	700—2 HRS.			700—5 HRS.			700—16 HRS.			750—2 HRS.			750—5 HRS.		
1.	8.19	9.69	7.88	7.75	9.62	7.41	7.31	9.22	7.49						
2.	8.18	9.85	7.74	8.04	9.88	7.74									
3.	8.58	10.12	7.53	8.24	9.59	7.32									
4.	7.16	8.86	7.78	7.24	8.73	7.48	6.95	8.64	7.11						
5.	7.90	9.69	7.71	7.62	9.45	7.60	7.28	8.85	7.50	7.63	8.94	7.32			
6.	8.67	10.00	8.04	8.45	10.00	8.00	8.24	9.80	7.85	8.52	9.92	7.98	8.19	9.86	7.92
7.	8.18	9.54	7.76	7.15	9.32	7.34	7.35	9.34	7.64						
8.	8.45	10.40	7.73	8.06	10.07	7.60									
9.	8.21	10.03	7.80	7.51	9.17	7.61	7.01	8.93	7.61						
10.	7.72	10.20	8.36	7.38	9.88	8.18	6.68	8.96	8.40						
13.	7.88	9.47	7.63	7.48	9.12	7.44	7.08	8.81	7.36						
14.	8.18	9.75	7.64	7.54	9.50	7.50	7.07	8.65	7.43						
15.	8.03	9.93	7.58	7.87	9.90	7.37	7.58	9.60	7.20	7.89	9.50	7.27	7.52	9.23	7.21
16.	8.68	11.10	9.43	8.02	9.80	7.36	8.01	9.80	7.35						
17.	7.10	9.00	7.44	6.78	8.53	7.37				7.16	9.03	7.38	6.85	8.43	7.27
18.															
19.	8.88	9.65	7.56	7.12	9.11	7.65									
20.	8.48	9.84	7.83	8.22	9.42	7.53									
Av.	8.15	9.83	7.84	7.73	9.53	7.66	7.37	9.20	7.55	7.80	9.35	7.50	7.52	9.17	7.47

TABLE 2.—Collaborative results—(Continued)

	750—10 hrs	800—2 hrs	800—5 hrs	800—16 hrs	850—2 hrs*
1.					
2.					
3.					
4.					
5.	7.75			7.14 8.97 7.68	7.48 9.45 7.96
6.	9.48 7.77	7.91 9.75 7.90	7.65 9.49 7.82	7.50 9.28 7.77	7.16 8.90 7.43
7.					
8.					
9.					
10.					
13.					7.10 8.81 7.11
14.					
15.					
16.					
17.		6.82 8.56 7.32	6.78 8.57 7.44		6.78 8.43 7.23
18.					
19.					
20.					
Av.					7.13 8.90 7.43 7.43

* At 850—5 hrs., Collaborator 6 obtained 7.39, 9.28, and 7.95, and Collaborator 17 obtained 6.90, 8.58, and 7.37.
At 800—2 hrs., Collaborator 5 obtained 9.85, 11.80, and 10.20.

The results submitted by the 18 collaborators who reported are presented in Table 2. Each result is the average of the duplicate determinations submitted by a collaborator. The data in each vertical column, that is the average results of all collaborators on each sample for each condition under which ash was determined, are averaged, and these averages are collected in Table 3 for comparison. A major portion of the data covers temperatures from 550° through 700° for 2, 5, and 16 hours. Each average under these conditions represents the results obtained by fifteen to eighteen different collaborators, the number being sufficiently large so that it probably has some statistical significance. Since some data

TABLE 3.—*Average of collaborative results on ash*

TEMP.	TIME	1	2	3
°C.	*hours*	*per cent*	*per cent*	*per cent*
550	2	9.34	12.21	10.93
	5	8.96	11.63	10.16
	16	8.78	11.14	9.38
600	2	8.66	11.33	9.79
	5	8.45	10.67	8.84
	16	8.23	10.34	8.50
650	2	8.43	10.52	8.74
	5	7.99	9.93	7.88
	16	7.72	9.58	7.73
700	2	8.15	9.83	7.84
	5	7.73	9.53	7.66
	16	7.37	9.20	7.55
750	2	7.80	9.35	7.50
	5	7.52	9.17	7.47
850	2	7.13	8.90	7.43

were obtained from collaborators who also ashed at 750° and 850°, the averages are also presented in Table 3, the results being the average of the work of three or four collaborators in each case.

In Table 3 certain rather definite trends are evident. In practically all cases for each length of time and for all samples, the percentage of ash decreases as the temperature increases. In addition to this, at each temperature an increase in the length of time of ashing produces a lower percentage of ash. The percentage of ash in Sample 2 is consistently higher than it is in Sample 1, as would be expected. Sample 3 was intermediate in ash level between Samples 1 and 2, although for conditions beyond 600° at 5 hours the differences between Samples 1 and 3 are not large. Increasing the temperature to 750° and 850° further reduces the ash percentage.

In general, the results presented in Table 3 might be anticipated. However, they do not indicate the set of conditions most suitable and most practical for ash determination. They raise the question, "What is the percentage of ash in these three samples?" In an endeavor to approach an answer to this problem, certain comparatively simple statistical studies were made, including the probable error of the mean (PEm) of these averages, which is presented in Table 4. The probable errors indicate that it would not be difficult to duplicate the averages in Table 3 within 0.1 per cent if this study were repeated in the case of Sample 1, and with Samples 2 and 3 for conditions beyond 650° at 5 hours. For Samples 2 and 3, from 550° at 2 hours through 650° at 2 hours, the con-

TABLE 4.—*Probable error of the mean of ash determination*

TEMP.	TIME	1	2	3
°C.	hours	per cent	per cent	per cent
550	2	.087	.102	.118
	5	.088	.153	.194
	16	.096	.172	.230
600	2	.095	.163	.238
	5	.070	.153	.226
	16	.077	.135	.197
650	2	.067	.123	.195
	5	.048	.055	.059
	16	.076	.088	.053
700	2	.081	.087	.075
	5	.081	.090	.079
	16	.091	.091	.074

sistency in the results obtained by different investigators is not so desirable as indicated by the probable error of the mean, which varies from well over 0.1 per cent of about 0.25 per cent.

The range in the results obtained by the different collaborators is also indicated in Table 5, where the difference between the maximum and minimum result obtained under each condition of ashing is tabulated. These differences between maximum and minimum results seem comparatively large, varying from slightly over 1 per cent to about 4 per cent. Here again it will be noted that the maximum ranges between collaborators are found in the case of Samples 2 and 3 under conditions ranging from 550° at 2 hours through 650° at 2 hours.

The Associate Referee also studied two or three methods of measuring the accuracy obtained by each collaborator. Each man was asked to make each determination in duplicate and to carry his results to the

second decimal place. The differences between duplicates for each condition under which ashing was done was determined for each one of the collaborators and is shown in Table 6. How well each collaborator was able to check his own results throughout this series of determinations by averages of the difference between duplicates for each condition of ashing is shown in Table 7. These averages vary from .04 to .64, with a fairly even distribution between these two values, as shown by the following series: .04, .07, .10, .13, .15, .15, .15, .16, .19, .20, .21, .24, .27, .29, .31, .44, .64.

In view of the range of results shown in Tables 3, 4, and 5, a second average of the differences between duplicates for all of the collaborators

TABLE 5.—*Maximum range between results obtained by collaborators*

TEMP.	TIME	1	2	3
°C.	hours	per cent	per cent	per cent
550	2	1.65	2.02	2.26
	5	1.58	2.55	3.66
	16	2.06	2.96	4.06
600	2	1.84	2.69	3.53
	5	1.48	2.55	3.71
	16	1.73	2.72	3.75
650	2	1.59	2.47	3.75
	5	1.11	1.13	1.12
	16	1.28	1.91	1.31
700	2	1.78	2.24	1.99
	5	1.93	1.96	1.93
	16	1.56	1.24	1.29

was made; it included mainly the results from 650° at 16 hours through 700° at 16 hours, together with those at 650° at 2 and 5 hours and 600° at 5 and 16 hours for Sample 1. These averages are presented in the second column of Table 7. The range of differences between duplicates was from .04 to .34. Those who obtained the greatest differences for the group as a whole showed a materially smaller result when the latter average is considered. A number of the remaining collaborators secured essentially as great an accuracy in the results at lower temperatures, so far as this is shown by the difference between the duplicate determinations, although in several cases improvement was shown.

From Table 6 averages were also made of the difference between duplicates for each condition of ashing. These were combined in Table 8 to indicate the facility with which the collaborators, as a group, may check their own results when determining ash under each of the specified conditions. While in general the variations between duplicates for Samples 2

TABLE 6.—Differences in duplicate results of collaborators

	COLLABORATOR NO.	550—2 hrs.			550—6 hrs.			550—16 hrs.			600—2 hrs.		
1. Adams	22	.02	.07	.01	.09	.05	.01	.29	.84	.51	.03	.05	.04
2. Becknell	30				.03	.15	.24	.20	.15	.23	.36	.69	.59
3. Brooke	2	.01	.33	.14	.08	.34	.20	.08	.33	.66	.14	.06	.23
4. Clulow	33	.35	.21	.18	.03	.05	.31	.22	.16	.12	.16	.05	.00
5. Geagley	9										.08	.22	.18
6. Grattan	10	.43	.06	.47	.42	.06	.48	.43	.06	.47	.18	.06	.42
7. Halvorsen	12	.17	.24	.36	.34	.20	.02		.08	.48	.04	.17	.33
8. Hand	13	.26	.19	.26	.33	.20	.02	.65	.19	.23	.13	.34	.33
9. Haskins	15	.51	.10	1.15	.34	.33	.42	.32	.04	.02	.33	.34	.04
10. King	17	.05	.52	.39	.19	.65	1.11	.34	.13	.87	.31	1.12	3.82
13. Montzheimer	34	.21	.31	.16	.10	.12	.20	.07	.12	.08	.03	.01	.05
14. Nixon	6	.05	.01	.05	.05	.40	.17	.33	.23	.00	.18	.42	.11
15. Randall	29	.34	.40	.06	.07	.02	.07	.04	.12	.16	.00	.02	.08
16. Struve	23	.03	.32	.00	.13	.14	.02	.23	.80	.96	.04	.28	.06
17. Tobey	24				.10	.09	.43	.07	.26	.21	.19	.49	.09
18. Walker	25												
19. Ziegler	27							.00	.02	.55	.33	.15	.12
Averages		.22	.23	.25	.16	.19	.27	.24	.23	.37	.21	.34	.42

TABLE 6.—*Differences in duplicate results of collaborators*—(Continued)

	600—5 HRS.			600—16 HRS.			650—2 HRS.			650—5 HRS.			650—16 HRS.		
1.	.04	.04	.01	.14	.05	.19	.02	.04	.01	.05	.06	.04	.21	.10	.21
2.	.04	.35	.24	.25	.15	.10	.22	.13	.48	.08	.08	.25	.03	.17	.20
3.	.11	.19	.01	.13	.24	.12	.29	.03	.08	.21	.19	.10	.20	.11	.11
4.	.01	.22	.12	.54	.02	.14	.14	.31	.07	.22	.13	.18	.03	.12	.19
5.	.16	.30	.12	.12	.65	1.22	.28	.15	.11	.17	.17	.04	.69	.15	.02
6.	.07	.22	.34	.18	1.28	1.45	.04	.54	.58	.02	.46	.75	.15	.65	.12
7.	.16	.01	.64	.04	.02	.16	.78	1.35	1.23	1.41	.04	.33	.04	.19	.13
8.	.13	.08	.19	.31	.17	.13	.23	.45	.13	.16	.17	.02	.01	.12	.25
9.	.09	.08	.09	.02	.87	1.58	.28	.10	.44	.12	.31	.28	.89	.45	.18
10.	.24	.40	3.25	.03	.02	.04	.10	.35	.96	.07	.37	.95	.01	.03	.05
13.	.09	.00	.07	.06	.17	.00	.01	.04	.04	.01	.02	.02	.06	.17	.26
14.	.35	.37	.62	.24	.35	.63	.21	.00	.01	.13	.38	.17	.08	.05	.04
15.	.00	.05	.16	.13	.22	.12	.02	.30	.08	.03	.10	.07	.03	.00	.43
16.	.11	.08	.58	.18	.32	.08	.32	.09	.03	.19	.72	1.04	.10	.12	.00
17.	.11	.33	.11	.15	.03	.10	.12	.16	.02	.19	.15	.09	.02	.19	.08
18.							.18	.34	.26	.17	.32	.32			
19.	.28	.05	.05				.07	.05	.07	.20	.28	.05	.20	.20	.15
Av.	.13	.18	.40	.16	.30	.39	.20	.30	.27	.19	.24	.27	.17	.18	.15

Table 6.—*Differences in duplicate results of collaborators*—(Continued)

	700—2 hrs.			700—5 hrs.			700—16 hrs.			750—2 hrs.			750—5 hrs.			750—16 hrs.		
1.	.08	.07	.03	.01	.01	.07												
2.	.14	.33	.14	.12	.23	.10	.14	.03	.09									
3.	.10	.22	.09	.14	.08	.17												
4.	.16	.06	.03	.03	.00	.20	.02	.16	.05									
5.	.31	.10	.03	.33	.17	.00	.01	.08	.00	.01								
6.	.33	.19	.17	.35	.18	.15	.34	.15	.15	.54	.03	.10	.46	.03	.08	.43	.04	.06
7.	.01	.54	.01	.26	.37	.51	.05	.24	.02									
8.	.29	.34	.31	.26	.22	.09												
9.	.02	.08	.08	.12	.06	.17	.04	.39	.26									
10.	.01	.10	.62	.11	.25	.70	.03	.03	.12									
13.	.04	.05	.06	.03	.05	.08	.03	.03	.03									
14.	.31	.20	.04	.00	.09	.16	.08	.18	.08									
15.	.00	.05	.13	.01	.01	.16	.06	.01	.13	.06	.01	.01	.03	.07	.03			
16.	.09	.19	1.43	.05	.15	.05	.10	.02	.25									
17.	.11	.21	.13	.09	.11	.13				.11	.03	.19	.00	.24	.18			
18.																		
19.	.23	.00	.13	.00	.08	.10												
Av.	.14	.17	.22	.13	.16	.17	.08	.13	.11									

TABLE 7.—*Average difference between duplicates for each collaborator*

COLLABORATOR	TOTAL	LIMITED
22	.04	.04
30	.24	.15
2	.15	.16
33	.15	.10
9	.15	.16
10	.31	.19
12	.44	.34
13	.20	.19
15	.19	.14
17	.64	.25
32	.29	.19
34	.07	.04
6	.19	.15
29	.10	.06
23	.27	.14
24	.16	.12
27	.13	.13

and 3 under conditions ranging from 550° at 2 hours to 650° at 5 hours may be somewhat greater than in the remainder of the cases, one might conclude from inspection of this table, in comparison with Tables 3, 4, and 5, that the individual collaborators checked their own results for the different conditions more satisfactorily or with a greater consistency than is the case when the results of the different collaborators are compared. However, in the opinion of the Associate Referee the average difference between duplicates, as indicated by Column 2 in Table 7 and also by Table 8, is materially larger than is desirable.

TABLE 8.—*Average difference between duplicates for each set of conditions under which ashing is done*

TEMP.	TIME	1	2	3
°C.	hours	per cent	per cent	per cent
550	2	.22	.23	.25
	5	.16	.19	.27
	16	.24	.23	.37
600	2	.21	.34	.42
	5	.13	.18	.40
	16	.16	.30	.39
650	2	.20	.30	.27
	5	.19	.24	.27
	16	.15	.18	.17
700	2	.14	.17	.22
	5	.13	.16	.17
	16	.08	.13	.11

It is anticipated that with the same method the experience of various laboratories would be the same as that in this laboratory, where an average of the differences between duplicates in ashing 70 samples selected at random from a regular run of control samples showed an average variation between duplicates of .060, very few results running above .10. Over a third of this group of samples of concentrated commercial feeding stuffs contained shell flour or calcium carbonate in some form or other.

The second method of determining the facility or accuracy with which the different collaborators obtained ash results under each of the specified conditions may be measured by a determination of the probable error of the average results obtained by each collaborator for each of the

TABLE 9.—*Probable error of the average of ash collaborators' results for each ashing condition*

TEMP.	TIME	1	2	3
°C.	hours	per cent	per cent	per cent
550	2	.337	.394	.455
	5	.354	.612	.778
	16	.406	.731	.975
600	2	.391	.672	.951
	5	.298	.648	.932
	16	.307	.541	.763
650	2	.285	.524	.826
	5	.196	.228	.241
	16	.314	.364	.217
700	2	.335	.358	.310
	5	.344	.330	.309
	16	.317	.314	.226

specified conditions. The probable error of a single determination (PEs), that is the probable error of each man's average, is presented in Table 9. The probable error of a single determination in this instance indicates that the average obtained by any one collaborator has a fifty-fifty chance of coming within the plus or minus range of the quantity in Table 9 of the average. Again, in the opinion of the Associate Referee, these results are greater than the allowable variation.

Considering Table 9, one might conclude from the results for Sample 1 that the accuracy of any one man's result for the different conditions of ashing was essentially the same, and also that these accuracies were essentially the same as those for Samples 2 and 3 for the conditions between 650° at 5 hours and 700° at 16 hours. However, the facility with which the different collaborators may determine the ash in Samples 2 and 3, containing comparatively large quantities of calcium carbonate, under conditions varying from 550° for 2 hours through 650° for 2 hours,

is decidedly less. The probable error of a single determination in this latter group varies from about .40 to nearly 1.0, indicating rather definitely that with the large amounts of calcium carbonate in the samples different collaborators may not be expected to obtain satisfactory ash results. Other forms of calcium carbonate may show less variation.

A rather simple comparison is made in Table 10, which presents the difference between the percentage of ash for these samples as shown in Table 3. In Table 10 the difference in the percentage of ash between Samples 1 and 2, 2 and 3, and 1 and 3 is shown. It will be noted here that the percentage of ash in Samples 1 and 3 is very nearly the same when comparison is made of the results obtained by ashing under conditions varying from 600° at 5 hours to 700° at 16 hours. It is believed that this

TABLE 10.—*Difference in amount of ash between three samples for each condition*

TEMP.	TIME	2–1	2–3	3–1
°C.	hours	per cent	per cent	per cent
550	2	2.87	1.28	1.59
	5	2.67	1.47	1.20
	16	2.36	1.76	.60
600	2	2.67	1.54	1.13
	5	2.22	1.83	.39
	16	2.11	1.84	.27
650	2	2.09	1.78	.31
	5	1.94	2.05	−.11
	16	1.86	1.85	.01
700	2	1.68	1.99	−.31
	5	1.80	1.87	.07
	16	1.83	1.65	.18

tends to confirm the observation that ashing conditions of 600° at 5 hours and beyond are more satisfactory for determinations on samples containing calcium carbonate.

It is also interesting to compare the results obtained in this laboratory on these samples when determinations were made by the method in use. Two gram samples are placed in a cold muffle at night, and the rheostat is set so that the furnace reaches a temperature of 650° and maintains this temperature throughout the night. These determinations were made in the regular course of control work and placed in the muffle with control samples. This was the first and only set of determinations made under these conditions. The results are as follows:

	1	2	3
	8.29	10.21	8.13
	8.44	10.26	8.14
	8.37	10.24	8.14
Av. difference	.15	.25	.01

In addition to the work suggested two collaborators made ash determinations at the different temperatures, placing the samples in a hot muffle and ashing for 2 hours. These results are presented in Table 11. In general it appears from these results that this method gives a per-

TABLE 11.—*Results obtained by two collaborators placing sample in hot oven*

TEMP.	TIME	1	2	3	CaCO₃
°C.	*hours*	*per cent*	*per cent*	*per cent*	*per cent*
550	2	9.69	12.47	10.25	
600	2	8.33	9.83	7.92	97.07
		8.92	11.44	10.23	97.32
650	2	8.18	9.60	7.56	92.40
		8.58	10.02	8.27	93.24
700	2	7.14	8.93	7.63	58.32
		8.27	10.06	8.31	62.64

centage of ash lower than is obtained under the same condition when the sample is put in a cold muffle, and results are more nearly equal to the results obtained when ashing is continued for a much longer period of time if the sample is put into a cold muffle.

TABLE 12.—*Percentage of moisture reported by collaborators*

COLLABORATOR	1	2	3
22	8.78	4.05	8.43
2	7.95	7.64	8.42
33	9.0	8.65	9.12
9	8.46	8.45	8.92
10	9.53	8.45	9.50
17	9.27	11.18	8.19
32	—	—	—
34	7.8	7.6	8.6
6	9.78	9.26	9.21
24	7.86	7.30	8.34
25	8.96	8.49	8.93
27	8.65	8.25	9.00

The collaborators were asked to determine the percentage of moisture in the samples at the time they were weighed out for the ash determination. The results are reported in Table 12. In view of the rather wide variations in the moisture determinations themselves no attempt was made to convert the ash results to the same moisture basis in all cases. It is possible that further work needs to be done on the moisture determination.

In view of the results presented in this paper a number of points seem evident to the Associate Referee.

(1) There is a decided need for a standardization of conditions under which ash determinations are made.

(2) The conditions to which the method should be standardized are not sufficiently evident.

(3) Further work may be needed on the standardization of the method for ash determination on samples that do not contain calcium carbonate as well as those that do.

(4) Especially where larger quantities of calcium carbonate are present the results suggest that the ashing should be done at a temperature of 650° or above for at least 5 hours.

(5) Because of the variation in results obtained by different collaborators, it is probable that a much closer adherence to the specified conditions is essential.

(6) The placing of the samples in a hot muffle and the use of methods such as the magnesium acetate and other modified methods should be investigated.

(7) It is recommended[1] that the collaborative work on ash determination in feeds both with and without calcium carbonate be continued.

TABLE 13.—*Coefficient of variations*

TEMP.	TIME	1	2	3
°C.	hours	per cent	per cent	per cent
550	2	5.35	4.79	6.17
	5	5.84	7.80	11.3
	16	6.85	9.73	15.4
600	2	6.69	8.79	14.4
	5	5.21	9.00	15.6
	16	5.54	7.75	13.3
650	2	5.00	7.38	14.0
	5	3.64	3.40	4.54
	16	6.02	5.64	4.15
700	2	6.09	5.40	5.85
	5	6.59	5.15	5.99
	16	6.36	5.05	4.42

TABLE 14.—*T test*

TEMP.	TIME	1	2	3
°C.	hours	per cent	per cent	per cent
550	2	.467	.546	.630
	5	.492	.851	1.080
	16	.569	1.024	1.365
600	2	.546	.938	1.321
	5	.417	.907	1.300
	16	.427	.752	1.056
650	2	.398	.733	1.156
	5	.274	.317	.336
	16	.438	.509	.302
700	2	.4679	.500	.433
	5	.482	.462	.433
	16	.428	.4271	.310

[1] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 50 (1939).

TABLE 15.—*Sigma*

TEMP.	TIME	1	2	3
°C.	*hours*	*per cent*	*per cent*	*per cent*
550	2	.500	.585	.675
	5	.524	.908	1.153
	16	.602	1.084	1.446
600	2	.580	.997	1.410
	5	.441	.961	1.382
	16	.456	.802	1.132
650	2	.422	.777	1.224
	5	.291	.338	.358
	16	.465	.540	.321
700	2	.497	.531	.459
	5	.510	.490	.459
	16	.469	.465	.334

REPORT ON MINERAL MIXED FEEDS*

By ALFRED T. PERKINS, *Associate Referee*, and B. W. BEADLE
(Kansas Agricultural Experiment Station, Manhattan, Kan.)

The major part of the Associate Referee's work was devoted to the tentative method for calcium oxide in mineral feeds. Some progress was also made on an iodine method, but at present the data do not warrant a report.

The calcium oxide method, *Methods of Analysis, A.O.A.C.*, 1935, 347, **44**, corrected in line 8 by changing "(pH 3.0−4.4)" to "(pH 2.5−3.0)" and in line 9 by changing "20–30 cc." to "10 cc.," was tested by the collaboration of over 20 chemists.

The analyses were made on three samples of high-lime feeds to which impurities had been added in order to give the method a severe test. Sample A consisted of pieces of limestone gathered at random from the surrounding terrain. The rocks were crushed and then ground in a Braun mill, equipped with steel disks, to pass an 80-mesh sieve. After sieving, the sample was thoroughly mixed, reground, and remixed, and after a third grinding and mixing was bottled in 4 oz. sample bottles. Sample B consisted of 56 per cent of Sample A and 44 per cent of impurities as $CuSO_4$, $Fe_2(SO_4)_3$, $KMnO_4$, Derby Soil, KI, $Al_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$, and Filtercel. The non-limestone portion was handled as a unit, ground to pass an 80-mesh sieve, thoroughly mixed, and then added to the limestone.

After the two portions had been mixed, the sample was reground and remixed three times and then bottled in 4 oz. sample bottles. Sample C consisted of 32 per cent of Sample A and 68 per cent of impurities such as $MgSO_4 \cdot 7H_2O$, Na_2HPO_4, $Fe_2(SO_4)_3$, bone charcoal, boiler scale, cottonseed meal, and wheat mill tailings. The same mechanical methods that were used in preparing Sample B were used for Sample C. Considerable supervised N.Y.A. labor was used in preparing the samples.

TABLE 1.—*Collaborators**

1. B. W. Beadle, 2. A. T. Perkins, 3. L. I. Miller, Kansas State College, Manhattan, Kan.

4. Oscar I. Struve, Eastern States Cooperative Milling Corp. Buffalo, N. Y.

5. H. R. Kraybill and P. B. Curtis, Purdue University, Lafayette, Ind.

6. Geo. E. Grattan and C. V. Marshall, Department of Agriculture, Ottawa, Canada.

7. E. R. Tobey and C. Harry White, Maine Agricultural Experiment Station, Orono, Me.

8. Richard O. Brooke, Wirthmore Research Laboratory, Malden, Mass.

9. Geo. H. Marsh and W. J. Marsh, Alabama Department of Agriculture and Industries, Montgomery, Ala.

10. Arthur L. Haskins, Pennsylvania State College, State College, Penn.

11. W. F. Hand, Mississippi State College, State College, Miss.

12. J. Frampton King, Department of Agriculture, Atlanta, Ga.

13. G. S. Fraps and J. F. Fudge, Agricultural and Mechanical College of Texas, College Station, Tex.

14. C. C. Zeigler, Swift and Company, Union Stock Yards, Chicago, Ill.

15. H. H. Hanson, State Board of Agriculture, Dover, Del.

16. W. B. Griem and Wenzel S. Thompson, Wisconsin Department of Agriculture and Markets, Madison, Wis.

17. Hugo W. Nilson and Arthur L. Fowler, Bureau of Fisheries, College Park, Md.

18. F. E. Randall, Cooperative G. L. F. Mills, Inc., Buffalo, N. Y.

19. T. H. Burton, Alabama Department of Agriculture and Industries, Auburn, Ala.

20. W. C. Geagley and M. M. Nasif, Department of Agriculture, Lansing, Mich.

21. L. S. Walker and E. F. Boyce, Vermont Agricultural Experiment Station, Burlington, Vt.

22. L. M. Nixon and H. D. Matheson, North Carolina Department of Agriculture, Raleigh, N. C.

* Collaborators are listed in the order in which the reports were received.

The three samples were sent out to 27 chemists who had signified willingness to collaborate. Twenty-one had reported at the time of writing this report, and these results have been considered in the discussion. Those reporting are listed in Table 1 with an identifying number.

DISCUSSION

The results submitted by the 21 collaborators reporting by the time the paper was prepared have been considered in the calculations and discussion.

The three sets of results obtained in the Kansas State College Laboratory were obtained entirely independently, except that the same apparatus and stock chemicals were used. Different sources of sodium oxalate were used as a standard for the potassium permanganate, but they had been compared with a sample of Bureau of Standards sodium oxalate. One of the three analysts was an undergraduate chemist.

TABLE 2.—*Collaborative results on calcium oxide and deviation from average**

COLLABORA- TION NO.	CaO (PER CENT)			DEVIATION FROM AVERAGE (PER CENT)			AV.
	SAMPLE			SAMPLE			
	A	B	C	A	B	C	
8	49.62	28.40	20.25	−0.56	−0.46	−0.33	−0.45
5	49.83	28.13	20.33	−0.35	−0.73	−0.25	−0.44
18	49.85	28.41	20.14	−0.33	−0.45	−0.44	−0.41
16	50.07	28.36	20.24	−0.11	−0.50	−0.34	−0 32
2	49.81	28.49	20.40	−0.37	−0.37	−0.18	−0.31
3	49.87	28.48	20.44	−0.31	−0.38	−0.14	−0.28
20	50.10	28.60	20.24	−0.08	−0.26	−0.34	−0.23
6	49.89	28.64	20.45	−0.29	−0.22	−0.13	−0.21
10	49.91	28.57	20.51	−0 27	−0.29	−0.07	−0.21
1	49.85	28.59	20.57	−0.33	−0.27	−0.01	−0.20
21	50.27	28.66	20.40	+0.09	−0.20	−0.18	±0.16
19	50.12	28.98	20.45	+0.06	+0 12	−0.13	±0.10
9	50.22	28.88	20.56	+0.04	+0.02	−0.02	±0.03
11	50.30	28.93	20.55	+0.12	+0.07	−0.03	±0.07
14	50.20	29.28	20.92	+0.02	+0.42	+0.34	+0.26
15	50.40	29.30	20.76	+0.22	+0.44	+0.18	+0.28
4	50.73	29.25	20.76	+0.55	+0.39	+0.18	+0.37
12	50.57	29.24	21.19	+0.39	+0.38	+0.61	+0.46
17	50.43	29.98	20.76	+0.25	+1.12	+0.18	+0.52
7	50.68	29.31	21.21	+0.50	+0.45	+0.63	+0.53
13	51.10	29.60	20.96	+0.92	+0.74	+0.38	+0.68
Av.	50.18	28.86	20.58	±0.29	±0.39	±0.24	±0.31
22	50.13	28.78	20.42	−0.05	−0.08	−0.12	−0.08

* Listed in order of average plus and minus deviations from average.

The average of all 21 analyses reported for each sample was made. No satisfactory method was apparent to eliminate certain figures that were rather far from the average.

Table 2 shows that, of the 63 individual calcium oxide reports received, seven varied from the average more than 0.5 per cent and 34 varied more than 0.25 per cent. All collaborators showed remarkable consistency in reporting high or low results. Of the 21 analysts reporting, 10 reported all

their results below the average, seven reported all their results above the average, and only four reported results above and below the average. These four had the lowest average deviations; *i.e.*, 0.03 per cent, 0.07 per cent, 0.10 per cent, and 0.16 per cent, and thus reported most nearly average results. The average deviation of all reports was 0.31 per cent, the maximum average deviation for one chemist was 0.68 per cent, and the maximum single deviation was 1.12 per cent. These figures show that a laboratory was apt to report high, low, or average results on all three samples. This seems to indicate that where laboratories do not check, there is a constant difference in the laboratories rather than a fault in the method.

It is believed that the main inaccuracies of the method are those that are inherent in any method and that the greatest improvement in the method can be made by the use of a uniform standard.

The results reported by the collaborators with the deviations from the average are given in Table 2.

COMMENTS OF COLLABORATORS

No. 13.—Suggests use of 100 cc. volumetric and 10 cc. aliquot and 0.07143 N $KMnO_4$ instead of 0.1000 N. Also suggests combustion temperature of 600°.

No. 15.—Checked Samples A by a CO_2 method and reported 50.41 per cent compared to 50.40 per cent.

No. 16.—Mentions method of adjusting pH caused difficulty as Samples A and B became alkaline on standing. Also reported somewhat higher results by method of Clifcorn, *This Journal*, 16, 240.

No. 17.—Filtered samples before taking aliquots. Reported use of 600° ± 10° for ignition.

RECOMMENDATIONS[1]

It is recommended—

(1) That preliminary work be continued on method for small amounts of iodine in mineral feeds.

(2) That study of the method for the determination of calcium in mineral feeds be continued. Additional collaborative work should be done and the potassium permanganate used should be standardized by the usual method in each laboratory and against a sample of sodium oxalate submitted with the samples. Results to be on moisture basis as received and on dry basis.

REPORT ON LACTOSE IN MIXED FEEDS

By D. A. MAGRAW (American Dry Milk Institute, Inc., Chicago, Ill.), *Associate Referee*

The collaborative work done during 1937 indicated that it was necessary to formulate a more satisfactory correction blank, determine the maxi-

mum and minimum fermentation temperatures, and ascertain whether alcohol can be used to eliminate the interference of peanut meal and low-grade tankage.

To study these phases of the method five samples of feed were hand mixed. They contained the more common feed ingredients used in commercial feed mixes. The dry skim milk used contained 51.4 per cent lactose as determined by the Munson and Walker method.

The five samples were then sent to the collaborators with the following instructions:

Directions for Collaborative Study on Determination of Lactose in Mixed Feed (1938)

The study involves the use of five samples, which will be investigated as follows:

I. *Maximum and minimum fermentation temperature.*
  a. Samples 1 and 2, follow Method I, which is attached, using a fermentation temperature falling in the range of 75°–80° F.
  b. Samples 1 and 2, follow Method I, using a fermentation temperature falling in the range of 80°–85° F.
  c. Samples 1 and 2, follow Method I, using a fermentation temperature falling in the range of 85–90° F.
II. *Alcohol as precipitating agent for possible elimination of interference by peanut meal and low-grade tankage.*
  a. All five samples, follow Method I, using a fermentation temperature of 80°–85° F.
  b. All five samples, follow Method II, using a fermentation temperature of 80°–85° F.
III. *Reporting of data*
  Report all determination, giving (1) mg. of CuO, (2) percentage of lactose as found by formula, (3) temperature of fermentation used on each determination, (4) any abnormalities, and (5) comments.

TABLE 1.—*Feed formulas*

SAMPLE NO.	1	2	3	4	5
Ground corn	27½	27½	22½	22½	22½
Fine ground oats	10	10	10	10	10
Wheat bran	10	10	10	10	10
Ordinary flour middlings	20	20	20	20	20
Alfalfa leaf meal	5	5	5	5	5
Fish meal	2½	2½	2½	2½	2½
Soybean oil meal	5	5	5	5	5
Limestone	2	2	2	2	2
Steamed bone meal	2	2	2	2	2
Salt	1	1	1	1	1
Meat scraps	5	5	—	—	—
Cottonseed meal	5	—	5	4½	—
Gluten feed	5	—	5	4½	—
Peanut oil meal	—	—	5	5	5
Low grade tankage	—	—	5	5	5
Dry skim milk	—	10	—	1	10

Method 1 was published in *This Journal*, 19, 605. Method 2 is changed as follows: An equal amount of 95 per cent ethyl alcohol is added to the filtrate after the water extraction, the precipitate formed is removed by centrifugalization, and the alcohol is boiled off and the solution washed into a 200 cc. volumetric flask. The appropriate changes are made in the final calculations to allow for changes in volume, and no blank is taken off.

TABLE 2.—*Various fermentation temperatures*

METHOD SAMPLE NO.	TEMP. OF FERMENTATION COLLABORATOR NO.	NO. 1 NO ALCOHOL 6 MG. LACTOSE BLANK			NO. 2 WITH ALCOHOL NO BLANK SUBTRACTED		
		75°–80° F.	80°–85° F.	85°–90° F.	75°–80° F.	80°–85° F.	85°–90° F.
		LACTOSE (PER CENT)					
1 Blank	1	.27	.19	0		.13	
						.00	
	2					.00	
	3	.75	.456	.42		.88	
	4		.0	.0			
			.0	.0			
	5		.72				
			.61				
			.15				
	6		.85	.26		.14	
			.0	.22		.14	
			.83	.27			
	7			.0		.0	
				.0			
2 5.14%	1	5.18	5.53	4.84		3.21	
	2					4.96	
						4.96	
	3	6.16	4.94	5.23		5.99	
		6.25	4.86	4.67		5.47	
	4		4.12	3.91			
			4.30	4.05			
	5		5.21				
			5.36				
			4.38				
	6		5.28	4.74		4.42	
			5.15	4.63		4.17	
				4.57		4.54	
	7		5.44			5.37	
			5.57				

RESULTS

The results of the analyses made by the seven collaborators are presented in Tables 2 and 3. The following are the collaborators:

1. A. M. Besemer, San Francisco, Calif.
2. A. H. Johnson, Baltimore, Md.
3. O. I. Struve, Buffalo, N. Y.
4. S. E. Danielson, Akron, Ohio.
5. P. B. Curtis, Lafayette, Ind.
6. W. S. Thompson, Madison, Wis.
7. L. L. Lachat, Minneapolis, Minn.

The results obtained by the collaborators are not sufficiently complete to warrant definite conclusions as to the effect on the recovery of the lactose of different temperatures of fermentation. This was undoubtedly due to work required. At the time the instruction sheets were sent out it was not realized that the amount of work involved was so great.

TABLE 3.—*Methods 1 and 2 at 80°–85° F. fermentation temperature on Samples 3, 4, and 5*

COLLABORATOR	SAMPLE 3 BLANK		SAMPLE 4 .51% LACTOSE		SAMPLE 5 5.14% LACTOSE	
	METHOD 1	METHOD 2	METHOD 1	METHOD 2	METHOD 1	METHOD 2
1	.65	0	1.09	.17	5.23	3.32
2		0		.34		4.68
		0		.30		4.71
3	.45	.52	.95	.98	5.68	5.85
	.32	.39	.82	.96	5.59	5.46
4	.02	.04			3.37	4.68
5	.07		.20		3.81	
	.29		.36		3.79	
			.07		3.89	
6	.98	0	1.26	.23	4.99	4.84
	.53	0	1.40	.41	5.14	4.76
	.95	0	1.52	.17		
7	0	.07	.63	.57	5.59	5.74
	0		.65		5.24	

The collaborative results are erratic and in many cases are too low, and they may be accounted for by the following comments made by W. S. Thompson:

No difficulty was encountered in clarification of samples. Variations in results might be explained by the poor quality of some of the yeast used. As large cakes of yeast are not necessary and are uneconomical to buy, and because it is not known under what conditions the yeast has been kept at the bakers (who usually sell the scraps of the cake left from baking), it appears that the method should be

worked out for the small cakes. These are always covered and kept cold and are more convenient to obtain than the other type of yeast. It seems that the yeast used may be the source of greatest error.****

Owing to this comment a letter was sent to the collaborators asking for information on the types of yeast used, and it was found that in many cases old baker's yeast or Fleischmann's small cake yeast (which

TABLE 4.—*A.D.M.I. results by use of Fleischmann's small cake yeast*

METHOD	NO. 1, NO ALCOHOL 6 MG. LACTOSE BLANK SUBTRACTED			NO. 2, WITH ALCOHOL NO BLANK SUBTRACTED		
TEMP. OF FERMENTATION	75°–80° F.	80°–85° F.	85°–90° F.	75°–80° F.	80°–85° F.	85°–90° F.
SAMPLE	LACTOSE (PER CENT)					
1 Blank			0 0		.12 .16 .10 .12	0 0
2 5.14%			4.93 5.06		4.75 4.79 4.87 4.73	4.68 4.46
3 Blank			0 0		.11 .09	.05 .05
4 .51%			.41–.65 .42–.55		.58 .51	.53
5 5.14%		4.72 5.10	4.77 4.67		4.59 4.84 4.77 5.07	4.56 4.69

can be bought in drug and grocery stores) was used. The effect on the recovery of lactose of the use of the small cakes of yeast was also verified in this laboratory quite by accident since the small yeast cakes were used during a period when the refrigerator was being repaired and it was not possible to keep the large one-pound cakes of Fleischmann's baker's yeast. These results are given in Table 4.

Table 5 gives the results obtained in the American Dry Milk Institute laboratories when only Fleischmann's fresh baker's yeast was used.

CONCLUSIONS

The following conclusions seem to be consistent with the results obtained this year:

(1) The fermentation temperature of 75°–80° F. with Method I is too low and gives higher results.

(2) 80°–85° F. is satisfactory with Method I except in the presence of peanut meal.

(3) 85°–90° F. with Method I appears to be too high in some cases, and therefore unsatisfactory.

(4) The fermentation temperature of 75°–80° F. with Method II is fairly satisfactory.

(5) 80°–85° F. with Method II is satisfactory.

(6) 85°–90° F. with Method II is fairly satisfactory.

(7) Peanut meal appears to present an interference with Method I, which is eliminated in Method II.

(8) The small Fleischmann yeast cakes usually give low results with the present correction factors.

(9) Old yeast, either baker's yeast or the small cakes, is unsatisfactory.

TABLE 5.—*A.D.M.I. results with Fleischmann's baker's yeast*

METHOD	NO. 1, NO ALCOHOL 6 MG. OF LACTOSE BLANK SUBTRACTED			NO. 2, WITH ALCOHOL NO BLANK SUBTRACTED		
TEMP. FERMENTATION	75°–80° F.	80°–85° F.	85°–90° F	75°–80° F.	80°–85° F.	85°–90° F.
SAMPLE	LACTOSE (PER CENT)					
1	.67	.26	.007	.34	.05	.15
Blank	.73	.26	.10	.00	.10	.0
	.74	.34		.38	.08	
	.47	.17			.11	
2	5.95	5.28	4.87	5.28	5.01	5.17
5.14%	5.48	5.15	5.16	5.20	5.05	5.13
	4.94	4.97		5.10		
	5.35			5.14		
3	1.01	.53	0	.09	.08	.20
Blank	.62	.52	0	.00	.10	.00
	.29	.25		.26	.11	.11
	.37	.25		.26		
4	1.01	1.11	.33	.78	.67	.55
.51%	1.21	1.11	.13	.78	.86	.53
	.78	1.19		.58		.75
		.58				.76
5	5.13	5.05	5.12	5.14	5.08	4.70
5.14%	5.37	5.12	5.17	5.03	4.63	4.75
	5.01			4.94	4.93	

It is recommended that the study be continued and that consideration be given to working out a correction factor whereby the Fleischmann's

small yeast cakes or some other small yeast cakes may be used and to the use of alcohol for elimination of interference from peanut meal and low-grade tankage.

No report on moisture in feeding stuffs was given by the associate referee.

REPORT ON BIOLOGICAL METHODS FOR ASSAY OF VITAMIN D CARRIERS

By W. B. Griem (Wisconsin Department of Agriculture and Markets, Madison, Wis.), *Associate Referee*

Last year collaborative work was conducted for the first time on the revised tentative A.O.A.C. method as adopted in 1936, *This Journal*, 20, 72. Thirteen collaborators assayed a sample of cod liver oil submitted to them. They were asked to interpret their own results. There was evidence of far better agreement in interpretation of results than agreement in actual ash values. In other words, the responses to vitamin D intake by the birds varied greatly among laboratories.

It was fortunate that a large number of the collaborators and others interested in this work were present at last year's meeting. Many hours were spent in informal discussion of the collaborative results and of the need for future work. It was quite generally agreed that the variations in the vitamin D reserves in the chicks were responsible for much of this variation in response. It was suggested that if all chicks were obtained from a few hatcheries maintaining controlled flocks, a far better agreement would be expected between laboratories.

In order to obtain information on several of the important details of the tentative method, a collaborative study was designed which was intended to clarify some of these factors. Because of several physical aspects of the work, it was deemed necessary to limit the activity to a relatively small group of collaborators. The collaborators are as follows:

(1) E. M. Bailey and R. B. Hubbell, Conn. Agr. Exp. Station, New Haven, Conn.
(2) L. E. Bopst, Maryland Regulatory Service, College Park, Md.
(3) F. D. Baird, National Oil Products Co., Harrison, N. J.
(4) R. F. Mann, White Laboratory, Newark, N. J.
(5) W. B. Griem.

In brief, the work consisted of a feeding trial conducted simultaneously in five laboratories. A basal rachitogenic ration and U.S.P. Reference Cod Liver Oil dilutions prepared by the Associate Referee were fed along with the collaborator's own basal and oil dilutions.

The chicks used in the trial were obtained from a hatchery making a

specialty of supplying experimental birds. The parent flock was confined indoors without benefit of sunlight. The flock received a pelleted mash as the only source of feed. The feed intake would be relatively equal between individuals. Such a flock would be closely culled for egg production. It was assumed that the chicks would be relatively equal, as nearly as practical, in vitamin D reserve. Each collaborator was supplied with birds impartially selected from the hatch.

Sufficient basal ration to furnish each laboratory with a supply for five groups was prepared by the Associate Referee in one batch. The calcium content was .88 of 1 per cent and the phosphorus content was .66 of 1 per cent. The calcium-phosphorus ratio was 1.33. This feed was carefully divided into five equal portions, and extreme precautions were taken to avoid any appreciable difference in the lots. Corn oil dilutions of the reference oil were prepared and subdivided. They supplied 5, 10, 15, and 20 U.S.P. or A.O.A.C. chick units per gram or supplied additions of this amount when 1 gram was added to 100 grams of basal ration. Instructions were submitted for the incorporation of these dilutions with the basal ration so that all laboratories would use an identical method.

At the four other laboratories there was prepared the basal ration from the sources of ingredients customarily used at the laboratories. These collaborators prepared their own comparable oil dilutions from the reference oil.

All oil incorporations for the various groups were made on the same day. Groups of sixteen birds were started instead of the minimum of ten. Collaborators were asked to darken the pens at night for the sake of uniformity.

Relative to the preparation of the bones for analysis, collaborators were asked to remove both tibiae, one to be used for individual ashing and the other for composite group ashing. A somewhat detailed method of preparing the bones was outlined to reduce irregularities to a minimum. A change in the present tentative procedure was incorporated in the instructions. This called for the extracting of the uncrushed bones rather than the crushed bones. It has become common practice in most laboratories to eliminate the crushing of the bones. They are far easier to handle uncrushed and they can be just as thoroughly extracted. The collaborators were asked to extract them for 30 hours with each solvent instead of the 20 hours specified. The Associate Referee is of the opinion that in most details a quite general agreement in procedure was obtained.

COLLABORATIVE EXPERIMENTAL RESULTS

The tabulation accompanying this report includes all the information requested by the Associate Referee with the exception of the individual tibia ash percentages. Instead of listing all of the individuals there is shown only the maximum and the minimum of each group.

Since the experiment did not involve the actual assay of a sample of oil, comparisons can only be made with ash values. With one exception of the possible 36, there was a distinct increase in tibia ash percentage with each increase in vitamin D. Collaborator No. 5, at the 20 unit level, obtained a value which was no higher than that obtained at the 15 unit level. The oil dilution used at this level was a replacement of the original dilution so that there is the possibility of an error in its preparation.

The regular increments in ash values is the encouraging part of the study. It was, however, anticipated that far better agreement between laboratories would have been obtained on that part of the experiment in which the subdivided basal and oil dilutions were used. The maximum differences in individual ash averages for each feeding level of the subdivided rations were 3.81, 8.16, 4.77, 4.89, and 8.90. The averages of Collaborator No. 4 were highest in the five instances, those of Collaborator No. 1 lowest in one instance, and those of Collaborator No. 2 and No. 5 each lowest in two instances.

In the second part of the experiment relating to the individually prepared rations the maximum ash differences were 5.27, 6.77, 8.89, 4.23, and 3.00. It is interesting to note that in this part of the experiment Collaborator No. 1 obtained a considerably lower response in calcification at the low vitamin D levels than was obtained on the subdivided rations, and Collaborator No. 2 obtained a considerably higher response. This indicates ration differences which both collaborators had previously observed* and which could not be explained by variations in total calcium and phosphorus content of the feed. It is suggested that the differences are due to variations in available phosphorus.

The variations in the individual ash percentages of all groups were large. In 24 of the 45 groups reported there were maximum differences of more than 10 per cent in the individual ash percentages. In 17 groups such differences exceeded 12 per cent, and in 4 groups such individual differences exceeded 15 per cent.

It could be expected that under controlled flock conditions as described previously, variations should be small. Narrowing the maximum spread with the groups tends to increase accuracy when relatively small numbers of birds are used. In the laboratory of the Associate Referee, greater differences between individuals have never been observed even when chicks from uncontrolled flocks have been used. Chicks such as were employed in this experiment may tend to give responses of a more uniform nature between different series of tests but they will not improve the accuracy of the method.

There is fair agreement in average chick weights for most vitamin D feeding levels with the exception of Collaborator No. 5 and a few other isolated instances. The chicks were all from the same source so that total

* Private communication.

feed consumption and insufficient vitamin D should in a large measure be responsible for the variations obtained. Total feed intake varies with laboratory conditions. It may be necessary eventually to specify such conditions as type of cage, temperature control, and period and intensity of lighting, if it can be shown that average weights seriously affect the accuracy of the test.

The collaborative work of last year, *This Journal*, 21, 607, indicated that composite ashing of the tibiae of the groups gives good agreement with the values obtained by averaging the individual tibia ash percentages. These present results again demonstrate the feasibility of such composite ashing. Only two of the forty-five groups indicate differences exceeding 1 per cent. In 27 instances the differences did not exceed .50 per cent. It should be remembered that individual variations were very large in this experiment. These differences are smaller than the differences that can usually be expected when duplicate groups are fed.

OBSERVATIONS

The basic soundness of the method is again demonstrated by the fact that there are quite definite increases in calcification with increased vitamin D intake.

As the individual variations in all groups were large, the Associate Referee is of the opinion that the most important problem to be studied for improving the accuracy of the method is that of reducing these variations. Until these individual variations within each group can be minimized it seems desirable to increase the size of the groups.

Comments from those using the method and others qualified in this field suggest revision of the basal ration so as to increase the manganese content and the ribo-flavin (vitamin G) content. There is a possibility of obtaining more uniform results between laboratories if the inorganic phosphorus content would be increased.

Composite ashing should be permitted and provision should be made to permit the extraction of uncrushed bones.

It seems desirable to rewrite the method at this time and to retain the fundamental features as now tentatively established.

RECOMMENDATIONS[1]

It is recommended—

(1) That investigational and collaborative work be continued.

(2) That the text of the tentative method be revised. The revision was published in *This Journal*, 22, 81.

No report on hydrocyanic acid in glucoside-bearing materials was given by the associate referee.

RATION	FEED AND OIL DILUTIONS PREPARED BY ASSOCIATE REFEREE					FEED AND OIL DILUTIONS PREPARED INDIVIDUALLY BY COLLABORATOR			
	COLLABORATOR					COLLABORATOR			
	1	2	3	4	5	1	2	3	4
Basal Negative Control									
No. of birds	14	16	14	15	18	15	16	14	16
Av. weight (g.)	112	120	128	125	105	116	114	128	126
Min. indiv. ash (%)	26.68	28.30	29.00	29.19	27.80	27.52	29.40	28.70	30.04
Max. indiv. ash (%)	32.46	36.58	36.60	42.72	37.80	33.32	37.89	36.80	40.67
Av. of indiv. ash (%)	30.60	31.25	32.30	34.41	32.67	30.07	32.63	33.20	35.34
Composite ash (%)	30.53	30.39	32.20	33.53	32.59	29.70	32.82	33.00	34.77
Diff. indiv.−comp.	+.07	+.86	+.10	+.88	+.08	+.37	−.19	+.20	+.57
Basal+5 A.O.A.C./100 g.									
No. of birds	15	15	15	16	16	15	16	15	15
Av. weight (g.)	116	142	136	144	122	110	128	133	135
Min. indiv. ash (%)	30.39	28.06	32.00	34.35	32.00	28.23	32.63	31.60	31.81
Max. indiv. ash (%)	40.23	37.56	40.00	47.15	40.40	36.50	45.75	49.40	44.26
Av. of indiv. ash (%)	34.61	32.73	36.10	40.89	35.34	31.59	38.36	36.60	38.29
Composite ash (%)	34.10	32.54	35.70	40.48	35.18	31.98	37.60	35.20	38.42
Diff. indiv.−comp.	+.51	+.19	+.40	+.41	+.16	−.39	+.76	+1.40	−.13
Basal+10 A.O.A.C./100 g.									
No. of birds	15	16	15	16	16	15	16	15	17
Av. weight (g.)	143	141	146	145	127	145	141	142	151
Min. indiv. ash (%)	31.94	32.17	30.50	34.17	33.30	27.20	36.91	32.60	37.94
Max. indiv. ash (%)	47.23	46.26	46.50	48.17	43.10	40.62	45.38	47.50	48.77
Av. of indiv. ash (%)	40.94	37.36	38.30	42.13	37.87	34.54	41.61	38.50	43.43
Composite ash (%)	40.92	36.48	37.50	41.60	37.79	35.51	42.77	38.60	43.17
Diff. indiv.−comp.	+.02	+.88	+.80	+.53	+.10	−.97	+1.16	−.10	+.26
Basal+15 A.O.A.C./100 g.									
No. of birds	16	15	15	16	16	15	16	15	16
Av. weight (g.)	151	148	147	148	121	142	151	149	162
Min. indiv. ash (%)	35.66	37.72	36.60	34.91	35.60	33.01	38.06	34.10	34.00
Max. indiv. ash (%)	47.10	44.85	48.90	49.55	46.60	47.88	48.31	47.70	49.74
Av. of indiv. ash (%)	42.75	41.92	42.00	44.77	39.88	40.58	42.02	42.00	44.81
Composite ash (%)	43.29	42.42	41.20	44.71	39.05	40.38	42.16	42.20	44.52
Diff. indiv.−comp.	−.54	−.50	+.80	+.06	+.83	+.20	−.14	−.20	+.29
Basal+20 A.O.A.C./100 g.									
No. of birds	16	15	12	15	15	15	15	15	17
Av. weight (g.)	151	183	156	155	129	173	148	161	147
Min. indiv. ash (%)	42.86	38.79	38.60	45.98	34.20	33.98	42.75	37.70	38.05
Max. indiv. ash (%)	50.12	46.26	49.70	51.76	45.60	47.92	47.91	47.30	49.34
Av. of indiv. ash (%)	46.52	43.44	43.30	48.20	39.30	43.05	45.47	44.60	46.05
Composite ash (%)	47.06	43.80	43.50	47.47	38.55	42.87	45.06	44.60	46.67
Diff. indiv.−comp.	−.54	−.36	−.20	+.73	+.75	+.18	+.41	+.00	−.62

REPORT ON FAT IN FISH MEAL

By R. W. HARRISON (U. S. Bureau of Fisheries, Seattle, Wash.), *Associate Referee*

At the last meeting, *This Journal*, 21, 618, data were given showing the rate of extraction and percentage of extract obtained with 12 different fat solvents when used on several samples of fish meal. The solvents tested were petroleum ether, hexane (pract.), heptane (pract.), ethyl ether, carbon bisulfide, cyclohexane, benzene, methylene chloride, trichloroethylene, chloroform, acetone, and 1.4 dioxane. With the exception of acetone, there was practically no increase in extract value after 4 hours' extraction. The gross extraction values for the various solvents increased in the above order, although solvents of similar general chemical structure gave quite similar results.

TABLE 1.—*Decrease in extraction values of various solvents due to oxidation of fish meal*

| | | SOLVENT EXTRACT VALUE | | | | DECREASE IN EXTRACT VALUE DUE TO OXIDATION | |
| | | MEAL NO. 3 SPECIAL SALMON MEAL | | MEAL NO. 4 COMMERCIAL SARDINE MEAL | | | |
SOLVENT	B.P.	AS RECEIVED	OXIDIZED	AS RECEIVED	OXIDIZED	MEAL NO. 3	MEAL NO. 4
	°C.	per cent	per cent	per cent	per cent	per cent	per cent
Petroleum ether	35–60	7.8	5.9	8.8	3.1	24.4	64.8
Hexane (pract.)	62–67	7.5	6.6	9.8	3.4	12.0	65.3
Heptane (pract.)	91–96	7.7	6.8	10.1	3.7	11.7	63.4
Ethyl ether	34.5	7.8	7.3	10.1	4.4	2.7	56.5
Isopropyl ether	63–69		7.1		4.1		
Carbon bisulfide	46.3	8.1	6.8	10.5	3.5	16.1	66.7
Cyclohexane	78–81	8.1	6.9	10.5	3.6	14.8	65.7
Benzene	79.6	8.4	7.5	11.0	6.1	10.7	44.6
Toluene	110–111				8.5		
Methylene chloride	40–41	8.8	7.7	11.4	5.3	12.5	53.5
Trichloroethylene	83–87	9.2	7.6	11.6	6.7	17.4	42.2
Chloroform	61.2	9.1	8.2	11.7	7.6	9.9	35.1
Carbon tetrachloride	76.8				4.2		
Acetone	56.1	9.0	7.9	11.9	6.7	12.3	43.7
Methyl isobutyl ketone	111–117		9.7		11.3		
1-4 Dioxane	91–101	11.4	9.9	16.2	12.9	13.2	20.4

Because of the limitations in laboratory equipment, it was impossible to continue an active study of the problem during the past year. However, two samples of meal, which had been placed in storage under conditions designed to accelerate oxidation, were analyzed to determine the effect of oxidation on the extract value given by the various solvents. These

values, together with the percentage decrease caused by oxidation, are given in Table 1. Generally speaking, all solvents tested were found to give definitely lower extract values after the meal had been oxidized. The unusually efficient recovery obtained with ethyl ether in the case of Meal No. 3 must be considered as particular to the sample of meal in question because in numerous other instances not reported here, ethyl ether extract has been found to decrease markedly as a result of oxidation. In fact, the present study has resulted from this limitation of ether extract as a measure of the true fat content of fish meal. If time permits, the work will be continued during the coming year.

REPORT ON BIOLOGICAL METHODS FOR COMPONENTS OF THE VITAMIN B COMPLEX

By O. L. KLINE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

In the Associate Referee's report of last year, written by C. A. Elvehjem, *This Journal*, 21, 622, further work with the chick method of assay for vitamin B_1 was described. The chick method is a prophylactic type of assay in which the incidence of polyneuritis in groups of chicks fed varying levels of the assay material is determined. The chicks are maintained on a vitamin-B_1-low diet consisting of a mixture of corn, wheat-middlings, and casein that has been autoclaved to destroy the B_1, with additions of salts, dried liver, and cod-liver oil. This method is specific for vitamin B_1 and is of particular advantage in the assay of low potency materials. However, in view of the limited experience with the chick method, further study was recommended by the Referee at that time.

During the past year this method has been used with good results in this laboratory. Characteristic polyneuritis was observed, and there was no difficulty in interpreting the results obtained with different levels of the material being assayed. No further collaborative work on this method has been carried out.

At last year's meeting this Associate Referee's work on a rat-curative procedure for vitamin B_1 assay was presented, *This Journal*, 21, 305. This method is essentially a modified Smith curative technic.[1] Changes in the diet allow for inclusion of the necessary components of the vitamin B complex, except B_1. This is accomplished by furnishing autoclaved peanuts and purified liver extract in addition to the usual supplements. By modifying the method of interpreting results a comparison of standard and unknown is made in the same animal by determining the length of curative response to each. In this procedure animals have consistently

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[1] U. S. Public Health Rpt., 45, 116 (1930).

developed uncomplicated polyneuritis in a 25–50 day depletion period and have been used for as many as 10–20 curvative responses when crystalline vitamin B_1 was administered.

During the past year this method was subjected to critical collaborative study in a number of laboratories by members of the U.S.P. Vitamin Committee, under direction of the Pharmacopoeia Revision Committee, and is now under consideration for inclusion in the Pharmacopoeia. In view of this consideration and since there is no advantage to be gained in duplication, no recommendation with respect to the rat-curative method will be made at this time.

A note to be published in the forthcoming issue of *Science*[1] describes briefly studies on the destruction of vitamin B_1 by the use of sulfite. This is an application of the original observation of Williams and associates,[2] who found that the vitamin is destroyed by cleavage of the molecule in the presence of sulfite. A vitamin-B_1-free basal diet of sucrose, purified casein, salts, fat, and cod-liver oil was used in these studies. Fifty grams of dried yeast was treated with 400 cc. of a 0.1 per cent solution of sodium sulfite, and sulfur dioxide was introduced until a pH 4.0 was reached. The material was allowed to stand in a tightly stoppered bottle for 5 days at room temperature, then dried at 60°C. Animals fed the basal diet supplemented with 15 per cent sulfite-treated yeast developed characteristic polyneuritis in 30–35 days. Animals that received the basal diet containing sulfite-treated yeast plus crystalline vitamin B_1 grew as rapidly as animals fed the basal diet supplemented with untreated yeast, showing average daily weight gains of 3.6 and 3.8 grams. Normal reproduction occurred in females in the group receiving sulfite-treated yeast plus vitamin B_1. These and other studies indicate that other components of the vitamin B complex are apparently unaffected by sulfur dioxide.

Sulfite treatment offers a practical and simple means of preparation of a diet satisfactory for the determination of vitamin B_1, and appears to have definite advantage over any procedure of purification previously used.

Modifications involving use of sulfite destruction may make for improvement in the chick method and may also give a more accurate rat growth procedure, either of which would be useful in the assay of low potency materials. This will require further study.

Methods for other components of the vitamin B complex have received considerable attention. Riboflavin has been measured by means of the chick test, the Sherman-Borquin rat-growth method, and by chemical means. Nicotinic acid determinations have been carried out with dogs. Rapid advances in this field have made these two substances, as well as vitamin B_6, available in crystalline form. With these in hand further

[1] *Science*, **88**, 508 (1939).
[2] *J. Am. Chem. Soc.*, **57**, 536 (1935).

improvements in assay methods may be expected. Since the attention of the Associate Referee has been focused on the vitamin B_1 determination during the past year, no recommendations with respect to methods for other B complex factors are offered at this time.

It is recommended[1] that further study of vitamin B methods be carried out, and that the recent developments in this field be utilized, with particular emphasis given to their application to low-potency materials.

No report on technic and details of biological methods, vitamin D carriers was given by the associate referee.

REPORT ON CAROTENE

By V. E. MUNSEY (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The study on carotene during the past three years has been on modifications of the Guilbert method. The essential difference in the modified methods is the use of petroleum ether for extraction of carotene in one case and ethyl ether in the other. The principle of the subsequent separation of the carotene and xanthophyll requires the removal of ethyl ether, another step that involves time and difficulty. The majority of the collaborators the past two years favored the petroleum ether modification, but since some preferred the ethyl ether procedure a modification in the method for removal of the ethyl ether was made, and this method was again included in the collaborative study this year. In this modification the ethyl ether is removed by addition of methyl alcohol, followed by evaporation of the mixture to a small volume, which results in removal of the ether and leaves the pigment in methyl alcohol rather than a nearly solid mass dissolved with difficulty, as in the procedure previously studied.

The collaborators this year were asked to analyze two samples of feed, No. 1, alfalfa meal, and No. 2, a mixed feed, by the petroleum ether procedure of Peterson and Hughes and the ethyl ether Fraps procedure with the slight modification just referred to. The choice of three procedures was submitted for measurement of the carotene concentration in solution, namely, the Peterson and Hughes spectrophotometric procedure, the Fraps 0.1 per cent potassium dichromate, and Russell's 0.036 per cent potassium dichromate. Some of the collaborators were also asked to analyze the samples by the Peterson and Hughes procedure, with and

without washing the petroleum ether free of alkali. Their results and the results of the Associate Referee show that leaving out the water washing has no effect, since the alkali is removed by the subsequent methyl alcohol extractions. The results obtained are included in Tables 1 and 2. The essential difference is that the results are lower by the modified Fraps procedure. In addition to the results requested, some of the collaborators reported results by methods used in their laboratories and others made their measurements by photoelectric colorimeters.

The following methods were studied:

Peterson-Hughes modification of Guilbert method.—This method was adopted as tentative and published in *This Journal*, 22, 79.

MEASUREMENT OF CAROTENE IN SOLUTION

No. 1. Peterson and Hughes Procedure.—After making the carotene solution up to volume, determine the concentration by the spectrophotometric method. For each determination make optical density measurements at wave lengths of 4500, 4700, and 4800 Å.U. Using the absorption coefficients calculated for beta carotene at these wave lengths, determine the carotene concentration for each wave length and take the average.

Extinction Coefficients

Wave Length Å	Skellysolve b. 60–70°	Petroleum Ether b. 40–60°
4500	238	243
4550		231
4700	200	207
4800	212	212

No. 2. Fraps Procedure.—Estimate the amount of carotene in the sample by comparing it colorimetrically against 0.1% $K_2Cr_2O_7$. Put the solution of the sample in the left-hand cup of the colorimeter and set the scale at 0.5 cm., 1 cm., 2 cm., 3 cm., or 4 cm., according to the amount of color present. Vary the depth of the dichromate solution in the right-hand cup until the density of color in both cups is equal, and make eight independent readings, putting them down in millimeters. Average the readings. Make the dichromate readings between 4 mm. and 12 mm. on the colorimeter. If a reading below 4 mm. can not be avoided, make it, but repeat the analysis with a larger sample.

By use of the table transform the millimeter depth of 0.1% dichromate in p.p.m. of carotene. Then calculate the p.p.m. of carotene actually in the sample by use of the formula. The formula and table were published in *This Journal*, 22, 79.

No. 3. Russell Procedure.—Estimate the amount of carotene in the sample by comparing it colorimetrically against 0.036% $K_2Cr_2O_7$. (The concentration of the solution before being compared colorimetrically should be about one-half as much as when compared against the 0.1% $K_2Cr_2O_7$.) Set the standard 0.036% $K_2Cr_2O_7$ at 10 mm. and match against the carotene solution by taking eight independent readings. The carotene solution readings should be between 7 and 14 mm. The 0.036% $K_2Cr_2O_7 = 2.06$ mg. per liter of carotene.

TABLE 1.—*Results on carotene on Sample 1 by the two methods referred to in this report (p.p.m.)*

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER[1]	0.1% $K_2Cr_2O_7$[1]	0.036% $K_2Cr_2O_7$[2]
		Procedure No. 1		
1		239	247	215
		228	241	215
		253	235	214
		257	230	212
		257	255	206
		266	267	214
		Av. 250	Av. 246	Av. 213
2			221	
			249	
			Av. 235	
3		248		252
		257		259
		254		256
		Av. 253		Av. 256
4		255	264	255
5	275			
6	246			240
	246			239
	Av. 246			Av. 240
7		266	224	222
		252	240	215
		Av. 259	Av. 232	Av. 219
8		262		258
		254		
		Av. 258		
9				215
10	227	217	207	189
11			225	202
12			235	224
13			250	
			248	
			Av. 249	

[1] *This Journal*, 20, 459 (1937).
[2] *Plant Physiology*, 10, 325 (1935).

TABLE 1.—(*Continued*)

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER	0.1% $K_2Cr_2O_7$	0.036% $K_2Cr_2O_7$
		Procedure No. 1—(*Continued*)		
14			245	261
			248	280
			—	—
			Av. 247	Av. 272
16[s]			245	242
			243	246
			—	—
			Av. 244	Av. 244
17[s]			221	
			221	
			—	
			Av. 221	
Av.	249	247	238	231
Max.	275	259	264	272
Min.	227	217	207	189
Range	48	42	57	83
		Procedure No. 2		
1				
2			203	
			201	
			—	
			Av. 202	
3		228		229
		227		232
		233		232
		—		—
		Av. 229		Av. 231
4		244	256	243
5	290			
6	237			231
	235			232
	—			—
	Av. 236			Av. 232
7		229	219	204
		239	214	198
		—	—	—
		Av. 234	Av. 217	Av. 201
8		247		
		238		
		—		
		Av. 243		

[s] Received too late to include in average.

TABLE 1.—(*Continued*)

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER	0.1% K₂Cr₂O₇	0.036% K₂Cr₂O₇
		Procedure No. 2—(*Continued*)		
9				219
10	220	219	220	208
11			225	191
12			235	226
13			225	
			227	
			—	
			Av. 226	
14				
16²				
17³			167.5	
			159.2	
			169.5	
			—	
			Av. 165.4	
Av.	248	234	226	219
Max.	290	244	256	243
Min.	220	219	217	191
Range	70	25	39	52

TABLE 2.—*Results on carotene on Sample 2 by the same methods used in Table 1 (p.p.m.)*

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER[1]	0.1% K₂Cr₂O₇[2]	0.036% K₂Cr₂O₇[2]
		Procedure No. 1		
1		85.2	90.0	79.0
		86.4	90.0	79.0
		82.5	84.3	73.8
		82.4	85.7	69.0
		86.7	89.6	71.8
		86.7	86.6	71.9
		—	—	—
		Av. 85.0	Av. 87.7	Av. 74.1
2			106	
			83	
			66	
			—	
			Av. 85	
3		83.5		82.2
		83.6		83.3
		83.4		84.3
		—		—
		Av. 83.5		Av. 83.3

[1] *This Journal*, **20**, 459 (1937).
[2] *Plant Physiology*, **10**, 325 (1935).

TABLE 2.—*(Continued)*

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER	0.1% K₂Cr₂O₇	0.036% K₂Cr₂O₇

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER	0.1% $K_2Cr_2O_7$	0.036% $K_2Cr_2O_7$
		Procedure No. 1—*(Continued)*		
4		86	81	83
5	92			
6	85.5			82.6
	85.8			82.5
	Av. 85.7			Av. 82.6
7		88.3	76.0	75.5
		85.1	82.2	71.5
		Av. 86.7	Av. 79.1	Av. 73.5
8		91		84
		95		
		Av. 93		
9				72.5
10	81.3	78.1	71.5	69.3
11			76.2	67.8
12			78.0	75.0
13			72.2	
			71.1	
			Av. 71.7	
14		87.5	91.5	
		85.0	91.5	
		Av. 86.3	Av. 91.5	
16ª			97.5	99.3
			98.3	101.6
			Av. 97.9	Av. 100.5
17ª			61.0	
			60.6	
			Av. 60.8	
Av.	86.3	85.4	79.6	77.8
Max.	92.0	93.0	87.7	91.5
Min.	81.3	78.1	71.5	67.8
Range	10.7	14.9	16.2	23.7
		Procedure No. 2		
1				
2			76	
			66	
			Av. 71	

ª Received too late to include in average.

TABLE 2.—(*Continued*)

COLLABORATOR	PHOTOELECTRIC COLORIMETER	SPECTROPHOTOMETER	0.1% $K_2Cr_2O_7$	0.036% $K_2Cr_2O_7$
		Procedure No. 2—(*Continued*)		
3		78.3		77.4
		80.6		77.7
		77.6		77.3
		Av. 78.8		Av. 77.5
4		85	79	81
5	100			
6	82.0			79.5
	82.0			79.3
	Av. 82.0			79.4
7		82.1	76.7	68.7
		84.7	81.0	70.8
		Av. 83.4	Av. 78.9	Av. 69.8
8		88		
		73		
		Av. 81		
9				75
10	81.7	79.8	81.7	70.7
11			75.8	63.5
12			78.0	75.0
13			70.0	
			67.5	
			Av. 68.8	
14			77.5	87.3
15[4]	47.5			
	49.5			
	48.2			
	Av. 48.4			
16				
17[4]			57.9	
			58.6	
			Av. 58.3	
Av.	87.9	81.6	76.3	75.4
Max.	100.0	85.0	81.7	87.3
Min.	81.7	78.8	68.8	63.5
Range	18.3	6.2	12.9	23.8

[4] Not included in average.

DISCUSSION OF RESULTS

Excluding those obtained by the photoelectric colorimeter, the results on both Samples 1 and 2 by both procedures are highest by the spectrophotometer and also show best agreement among collaborators, as was the case the past two years. The results by 0.1 per cent potassium dichromate are somewhat lower and show less agreement, but they are in better agreement, on the whole, than those by the 0.036 per cent potassium dichromate. The results with the 0.036 per cent potassium dichromate are lowest and they show a greater spread. The results by the modified Fraps procedure are the lowest. The results by the photoelectric colorimeter this year, as in previous years, indicate satisfactory agreement with the results by the spectrophotometer.

Under directions for use of 0.036 per cent potassium dichromate it is necessary to work within a very limited range of concentration, since the carotene reading should be between 7 and 14 mm., with the potassium dichromate reading set at 10 mm. On the basis of this limitation and the results obtained in these studies it seems preferable to choose the 0.1 per cent potassium dichromate as reference standard, but for greater reliance it should be standardized by several chemists by comparison against pure beta carotene and the average value taken, since the values obtained are too low, according to several of the chemists, which fact is confirmed by collaborative results.

No collaborative work was done with the neutral wedge photometer since none was available to the collaborators. However, the Associate Referee obtained precision comparable with the spectrophotometer, and the results agree well with those obtained by other means of measurement.

On the basis of these collaborative studies the Peterson and Hughes extraction procedure and measurement of carotene in solution by the spectrophotometer and the photoelectric colorimeter seem satisfactory for carotene determination. In the absence of these means of measurement, the 0.1 per cent potassium dichromate may be used.

The carotene reported by the present extraction procedures may be considerably in error, due to varying amounts of impurities measured as carotene. The Bureau of Dairy Industry has done considerable investigating on the amounts of these impurities, which have been determined by an adsorption procedure, with magnesium oxide used as an adsorbing agent. The amount of these impurities may be around 30 per cent, depending on the type of material, being much greater on low-grade hays than in the case of fresh-cut grasses, which may be very low. In view of the existence of this varying amount of impurities measured as carotene by the methods studied the past few years it would seem logical to give consideration to the application of the adsorption principle. Theoretically, such a plan seemed desirable, but as a practical routine operation for this year's study it did not seem advisable at this stage of development. The

application of the chromatrographic principle requires special equipment and experience. Its application is difficult enough from a qualitative standpoint and even more so from the quantitative aspect. The adsorption agent must be activated by specially controlled conditions and be packed in the adsorption tower in such a way that filtration will not be too slow and at the same time not too loose to cause channeling. After the solution is poured on the adsorbing agent the chromatogram must be developed, followed by removal of the different zones and elution of the adsorbed pigment. The reference, "Die Chromatographische Adsorptions-methode," Wien, 1937, by L. Zechmeister and L. V. Cholnoky, gives valuable information on this subject. Also in a recent reference Hoppe-Seyler,[1] using aluminum oxide as adsorbing agent for chromatographic adsorption analysis of small amounts of carotenoid, gives further evidence to indicate the possibility of the application of this principle. It would seem possible to make a direct adsorption of the solution containing the total carotenoid pigments without first removing the xanthopyll by use of the aqueous methyl alcohol. Further work on this subject of carotene determination should be devoted to the development of a method based on the adsorption principle.

Appreciation of the generous cooperation of the following collaborators is herewith expressed:

G. C. Crooks, Burlington, Vt.
D. S. Binnington, Board of Grain Commissioners, Winnipeg, Man.
J. M. Kniseley, Seattle, Wash.
Virgil Wodicka, Ralston Purina Co., St. Louis, Mo.
W. J. Peterson, Manhattan, Kan.
H. Boeddeker, Rossford, Ohio.
R. O. Brooke, Malden, Mass.
S. M. Greenberg, College Station, Tex.
C. H. Haurand, Bayonne, N. J.
W. Zimmerman, Geneva, N. Y.
F. Kopko, Geneva, N. Y.
Lyle Swift, Lafayette, Ind.
F. E. Randall, Buffalo, N. Y.
O. I. Struve, Buffalo, N. Y.
R. W. Caldwell, Davis, Calif.
A. J. Soderberg, Davis, Calif.
H. R. Bicknell, Sacramento, Calif.
A. L. Haskins, State College, Pa.

RECOMMENDATIONS[2]

It is recommended—

(1) That no more collaborative work be done on the Peterson-Hughes method for the present.

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[1] *Z. physiol. chem.*, 253, 40 (1938).
[2] For report of Subcommittee A and action by the Association, see *This Journal*, 22, 50 (1939).

(2) That the Peterson-Hughes method be adopted as a tentative method for the determination of carotene, and that the spectrophotometer be used or the 0.1 per cent potassium dichromate reference standard, preferably the former.

(3) That the potassium dichromate standard be rechecked by several chemists against pure beta carotene and the best conditions for accurate application be established before further collaborative work is done involving the use of this reference standard.

(4) That study of the application of the neutral wedge photometer and the photoelectric colorimeter be continued.

(5) That study be made on the application of a quantitative adsorption procedure for the determination of carotene.

REPORT ON MANGANESE IN STOCK FEEDS*

By JOHN B. SMITH, *Associate Referee*, and E. J. DESZYCK
(Agricultural Experiment Station, Kingston, R. I.)

Manganese deficiency apparently is an important cause of perosis, or slipped tendon, in chicks, and the use of various inorganic manganese compounds as mineral supplements is becoming a common practice. Several compounds have been found effective, notably the sulfate, the carbonate, oxides, certain manganese ores, etc. Rhodonite, a silicate ore, and rhodochrosite, a carbonate ore, proved less satisfactory. The topic has been summarized, very recently, by Schaible, Bandemer and Davidson.[1]

In attempting to write an official method for manganese in feeds the writers recognized that knowledge of the subject is increasing rapidly, but that the background is far from complete. Doubtless further work will show that some supplements are more effective than others. At the moment, however, the demand seems to be for a simple, rapid, comprehensive method for manganese, rather than for differentiation among compounds. The method proposed includes all acid-soluble forms of manganese in the ash of feeds and it seems unlikely that compounds excluded by this treatment are of nutritional value. It is more probable that some of the less valuable forms may be included. The method does not differentiate between manganese added as a supplement and that in other ingredients.

Correspondence with the collaborators who have had experience with this work, led to the selection of the colorimetric potassium periodate method for measuring the small quantities of manganese involved. This

well-known procedure was published originally by Willard and Great-house,[2] and has been reviewed comprehensively by Smith.[3] The details were selected from procedures that were in use by the collaborators. Perhaps it is well to note that the method is intended for measuring small quantities of manganese, and may not apply to the analysis of manganese salts and other carriers.

The procedure was published in *This Journal*, **22**, 78.

Six samples, based on two formulas popular in this region, were sent to collaborators. Manganese sulfate and manganese carbonate were added in the customary proportions and the sulfate in a considerably larger proportion. The common practice is to use about four ounces of these compounds in a ton of feed. It would seem almost impossible to mix such a small quantity commercially with sufficient accuracy to permit satisfactory sampling. In making up these samples, the manganese carrier was mixed thoroughly with another ingredient, and then combined with the remainder of the mixture by prolonged mixing. The samples were then ground to pass a 1 mm. sieve and again mixed very thoroughly. Despite storage of the ingredients during a very wet period before mixing, the ingredients appeared normal and mixed readily. However, moisture must have increased from exposure during the long mixing at a period of high humidity, for the samples showed abnormally high moisture and a little mold upon standing tightly sealed. For this reason, all results were calculated on the oven-dry basis in accordance with the moisture results reported by each collaborator.

Results for manganese are expressed in this report as parts per million. In work that is entirely scientific, the designation of units is not so important as are determinations intended for the layman. Guarantees and determinations as percentages would appear as small decimals, difficult to comprehend. Whole numbers are easier to compare. Milligrams per cent and milligrams per kilogram mean little to the feeder and require too much printing on tags. Ounces per ton is understandable, but would be a figure of low magnitude and require too much printing. The writers prefer parts per million abbreviated to p.p.m. It is the same type of unit as percentage, is easily translated into percentage units, and results are whole numbers within the limits of accuracy of the method. In accord with the trend in similar work, results are expressed as Mn rather than as manganese oxides.

The periodate colorimetric method for manganese appears twice among the methods of this organization, *Methods of Analysis*, A.O.A.C., 1935, XII, **13**; XXVI, **17**, but in the judgment of the writers these procedures are so different from the procedure presented here that a separate section in the publication is justified. The Associate Referee is also responsible

for a method for manganese in fertilizers, *This Journal*, **22**, 270. The procedure to be remembered eventually is similar to that for feeds and will be written to conserve space in the chapter on fertilizers.

TABLE 1.—*Description of samples and collaborative results, calculated as p.p.m. Mn on the oven-dry basis*

COLLABORATOR	SAMPLES*					
	A	A-1	A-2	B	B-1	B-2
H. Boeddeker, Larrowe Milling Co. Photoelectric colorimeter	45	92	151	46	88	106
Richard O. Brooke, Wirthmore Research Laboratory Photoelectric colorimeter	47	93	142	48	94	102
E. J. Deszyck Duboscq type colorimeter	50	104	153	49	91	112
Arthur L. Haskins, Penn State Coll. Duboscq type colorimeter	53	100	159	56	100	112
Oscar I. Struve, Eastern States Coop. Milling Corp. Duboscq type colorimeter	52	102	149	51	102	111
F. E. Randall, Coop. G. L. F. Mills, Inc. Duboscq type colorimeter	55	101	151	62	104	115
Average	50	99	151	52	97	110
Calculated	—	102	148	—	101	108

* *Sample A.*—Yellow corn meal, flour middlings, ground oats, dried skimmilk, alfalfa leaf meal, meat scraps, fish meal, wheat bran, limestone, salt, dicalcium phosphate, cod-liver oil.
Sample A1.—Sample A plus 52 p.p.m. Mn in $MnSO_4$.
Sample A2.—Sample A plus 98 p.p.m. Mn in $MnSO_4$.
Sample B.—Ingredients as in Sample A, but different proportions, without wheat bran and $CaHPO_4$.
Sample B1.—Sample B plus 49 p.p.m. Mn in $MnSO_4$.
Sample B2.—Sample B plus 56 p.p.m. Mn in $MnCO_3$.

Results submitted by collaborators appear in Table 1. The analysts had had previous experience with similar procedures, and the results are remarkably consistant. This is especially true, in view of the minute quantities of manganese involved, possible variation in sample uniformity, and unavoidable errors for colorimetric methods. Reports in the literature and experience with the method show that the ratio between acidity and manganese concentration is important, but the method as written appears to control this factor within the necessary limits.

In the judgment of the Associate Referee, the results submitted justify recommendation of the procedure as a tentative method. Minor changes

to avoid the time lost in evaporation after filtration of the insoluble residue, and the use of potassium nitrate with the acid mixture to aid in the destruction of the last traces of organic matter are possible, and have worked well in this laboratory. These should be tried collaboratively next year, also different feed formulas, and other carriers such as pyrolusite.

The writers express their appreciation of the advice received from several investigators prominent in the field of poultry nutrition, as well as that of the collaborators listed in this report.

It is recommended[1] that the method presented by the associate referee for the determination of manganese in grain and stock feeds be adopted as tentative, and that the study be continued.

REPORT ON ADULTERATION OF CONDENSED MILK PRODUCTS AND COD LIVER OIL

By P. B. Curtis (Department of Agricultural Chemistry, Purdue University, Lafayette, Ind.), *Associate Referee*

At the 1937 meeting of this Association the Associate Referee on Stock Feed Adulteration presented a method for the detection of starch or starchy materials in condensed milk products. Due to the simplicity and short time required to make the test it was deemed advisable to conduct a collaborative study of the method. This was done the past year with six laboratories participating in the work.

On June 23 four samples of condensed skimmed milk, three of which contained known amounts of cornstarch, were sent to the collaborators with the request that the samples be tested for starch according to the method outlined last year, and published in *This Journal*, 21, 596. A request was also made that the samples be listed in order of their predominance in starch content as revealed by the test.

A summary of the results is given in the following table.

Detection of starch in condensed milk products

COLLABORATOR	SAMPLES			
	1	2	3	4
1	++	—	+	trace
2	++	—	+	—
3	++++	—	+	trace
4	++	—	+	—
5	+++	—	++	+
6	+++++	—	+	—
Starch content (as prepared)	5%	none	1%	0.1%

The results (Table 1) show that the samples as prepared had the following composition: Sample 1, condensed skimmed milk containing 5 per cent starch; Sample 2, condensed skimmed milk with no starch added; Sample 3, condensed skimmed milk with 1 per cent starch; and Sample 4, condensed skimmed milk with 0.1 per cent starch. While all the collaborators agreed on the relative quantities of starch present some of them reported no starch in Sample 4, which was prepared to contain 0.1 per cent starch. This discrepancy may be due to the difficulty involved in obtaining a uniform mixture of the condensed skimmed milk with the small amount of starch added.

When the samples were sent out each collaborator was asked to submit any comments or criticisms of the method. Some of the comments received follow:

Analyst 3.—The method can be worked just as satisfactorily on the dried milk product as on the condensed milk product. I would recommend that the method be brought forward with a view towards making it official.

Analyst 4.—Suggests the following procedure in order to eliminate the greenish effect sometimes produced in the spot plate test: Dip a starch-free smooth texture filter paper into the I-KI test solution, or drop some of the test solution on the paper. Allow this paper to dry to apparent dryness in the air or in an air oven (at first while moist and heavy with iodine the paper is dark brown, but on drying the color becomes yellowish brown). Allow a drop or two of the suspected solution, prepared as indicated in the method, to fall upon the test paper. In the presence of much starch a clear blue spot will appear on the paper, free from excess iodine or other colored solution.

Analyst 5.—Likewise found that the spot plate test on Sample 4 gave a greenish brown color. He suggests that the iodine solution be added to the milk solution in a test tube rather than on the spot plate.

Analyst 6.—Believes that the method which has been in use for a number of years in his laboratory is much better adapted to the detection of starch than the one proposed. A brief outline of this method is as follows: Place a small amount of the sample on a slide, add one drop of the I-KI solution, mix well, and cover with a cover-glass. Examine under a microscope. In semi-solid or condensed milk products the individual starch grains are easily noted while in the dried materials the blue or purplish color is apparent.

In view of these various comments and criticisms the Associate Referee recommends[1]—

(1) That further study be made on the detection of starch or starchy materials as an adulterant of condensed milk products.

(2) That a study be made of methods for the detection of adulteration of cod liver oil.

CONTRIBUTED PAPERS

DETERMINATION OF AMINOPYRINE IN THE PRESENCE OF ANTIPYRINE AND CAFFEINE[1]

By F. C. Sinton, New York, N. Y., and F. A. Rotondaro,
Philadelphia, Pa. (U. S. Food and Drug Administration)

Methods for the determination of aminopyrine in mixture with antipyrine are given in the literature, but they depend essentially on titration or colorimetric procedures. During the course of regulatory work the writers were given a mixture of aminopyrine, antipyrine, and caffeine, and as a result of experimental work devised a method for the separation of the aminopyrine from the antipyrine and caffeine by means of extraction from a solution of controlled acid strength. It was found that from a 3.5–5 per cent solution of sulfuric acid, by weight, antipyrine plus caffeine could be quantitatively extracted with chloroform. On being made ammoniacal subsequently, the aminopyrine was quantitatively removed. It was also found that on saturating with anhydrous sodium sulfate the limits of acid strength necessary for quantitative separation were raised and broadened.

Determinations were made on a solution containing in 100 cc. 3 grams of aminopyrine, 2 grams of antipyrine, and 0.5 gram of caffeine. The following procedure was used:

Transfer to a separator a 10 cc. sample, add 5 cc. of the acid, and extract with 25 cc. portions of $CHCl_3$. Wash the extracts in a second separator with 5 cc. of 3.5% H_2SO_4. After making five extractions, test the next for complete extraction. Evaporate the $CHCl_3$, dry the residue at 80°–100° C. for 10 minutes, and weigh. Transfer the wash water to the original separator, make ammoniacal, and extract the aminopyrine with $CHCl_3$. Evaporate the combined $CHCl_3$ extracts, dry at 80°–100° C. for 10 minutes, and weigh.

The procedure involving the use of anhydrous Na_2SO_4 is the same except that preliminary to the $CHCl_3$ extraction the solution is saturated with the Na_2SO_4.

It is to be noted that the strength only of the 5 cc. of acid added has been determined. Since this is diluted with 10 cc. of water, the resulting solution is approximately one-third the strength, at least where the acid is low in concentration.

The results obtained show that antipyrine and caffeine are quantitatively removed from a sulfuric acid solution of 3.5–5 per cent. If the concentration is much less, aminopyrine is partly removed; if the concentration is increased, the antipyrine tends to be held in the acid. The same separation can be made on adding sodium sulfate to saturation, but the concentration of the acid appears to be less critical. The 5 cc. of acid added to 10 cc. of water must, however, be 30 per cent or more, since in

weaker strengths the aminopyrine tends to be thrown out of the acid. The sodium sulfate appears to depress the ionization of the acid and a stronger acid strength must be used than in the case of the simple acid.

RESULTS

10 cc. solution, no sodium sulfate

NUMBER	STRENGTH OF THE 5 CC. OF ACID BY WT.	ANTIPYRINE AND CAFFEINE (5 EXTRACTIONS)	RECOVERY	AMINOPYRINE	RECOVERY
	per cent		*per cent*		*per cent*
1	1.7	0.2696	107.8		
2	5	0.2595	103.8		
3	10	0.2501	100.0		
4	10	0.2487	99.5		
5	10	0.2514	100.7	0.2994	99.8
6	10	0.2496	99.8	0.3001	100.0
7	10	0.2503	100.1	0.2990	99.7
8	10	0.2504	100.2	0.2998	99.9
9	10	0.2496	99.8	0.3005	100.2
10	15	0.2486	99.4	0.2969	99.0
11	21.4	0.2396	95.8		
12	30	0.2211	88.4		
13	60.8	0.0725	30.1		

10 cc. solution, anhydrous sodium sulfate to saturation

NUMBER	STRENGTH OF THE 5 CC. OF ACID BY WT.	ANTIPYRINE AND CAFFEINE RECOVERY	AMINOPYRINE RECOVERY
	per cent	*per cent*	*per cent*
1	10	111.5	
2	15	114.2	
3	22.5	103.5	96.3
4	22.5	104.5	95.8
5	30	101.9	98.6
6	30	100.7	96.7
7	30	101.4	97.0
8	45	100.9	98.4
9	60	100.7	99.2
10	60	99.0	98.6
11	60	99.2	98.5

The aminopyrine residues obtained in both procedures had melting points generally within the U.S.P. range of 107°–109° C., showing a complete separation. In a few cases when the simple acid procedure was used, the melting point was a degree or two low, but still showing the product practically pure.

CONCLUSION

The method presented effectively separates aminopyrine from antipyrine and caffeine. The same method of procedure might presumably be applied to other combinations containing aminopyrine.

DETERMINATION OF ARSENIC IN SOIL TREATED WITH ACID LEAD ARSENATE

By LOUIS KOBLITSKY (Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, Washington, D. C.)

For several years acid lead arsenate has been used at the rate of 1,500 pounds per acre to free the soil of the immature larvae of the Japanese beetle, *Popillia japonica* (Newm.).[1] Because it is necessary to maintain this dosage to assure their elimination and permit certification of plants for shipment outside the area infested with the Japanese beetle, a method of analyzing soil to determine the arsenical concentration had to be developed.

It was believed that the method should involve removal of the arsenic from the soil, and furthermore, that it should detect from a trace up to 0.06 per cent of arsenic in the soil and lend itself to speedy manipulation, because of the large number of samples to be analyzed in a short time.

EXPERIMENTAL

To avoid the interference of some of the constituents of the soil, both a partial digestion with nitric acid[2] and a fusion with sodium carbonate and potassium nitrate[2] were investigated. The soil was filtered or the melt dissolved to bring the arsenic into a solution.

Gravimetric methods for evaluating the arsenic were not investigated, owing to the general slowness of such procedures. The volumetric procedures investigated, namely, those involving titration with potassium iodate or iodine,[3] require the arsenic to be in the reduced state. After its removal from the soil the arsenic can be reduced by distillation from a hydrochloric acid solution and the use of hydrazine sulfate and sodium bromide as reducing agents,[4] or by heating with potassium iodide in an acid solution.[2]

Combinations of the methods mentioned above were tried, but none was completely satisfactory. Because a large sample is necessary to obtain a measurable quantity of arsenic, the fusion procedure required too much time and attention to obtain a satisfactory melt. The partial digestion method was simpler and faster, but an equilibrium was reached wherein

[1] U. S. Dept. Agr., Plant Quarantine and Control Admin. Service and Regulatory Announcement No. 100, 1929, pp. 133–134.
[2] Scott, W. W. *Standard Methods of Chemical Analysis*, 3rd ed., 1922, Vol. 1, pp. 34–55.
[3] Jamieson, G. S. *Ind. Eng. Chem.*, 10, 290–292 (1918).
[4] Graham and Smith, *Ind. Eng. Chem.*, 14, 207–209 (1922).

not more than 90 per cent of the arsenic could be recovered. It was thought that if the digestion were carried to completion and the organic matter in the soil destroyed complete recovery would be possible. Attempts were made to rid the soil of organic matter by using (a) nascent chlorine liberated from potassium chlorate,[2] (b) liquid bromine,[2] and (c) carbon tetrachloride and potassium bromide,[2] and also by taking the soil to dryness three times with nitric acid.[5] Although the results obtained by these methods were satisfactory, the procedures were too complicated and time-consuming.

With the idea of reducing and removing the arsenic as soon as it goes into solution, direct distillation of the soil from a hydrochloric acid solution with hydrazine sulfate-sodium bromide solution as the reducing agent was tried. In following this procedure there was a tendency for the contents of the distilling flask to boil over at the outset of the distillation, but this difficulty was finally overcome by careful regulation of the temperature. Moreover, instead of a sharp blue color, various shades of purple were obtained in titrating some samples with iodine. Use of the sodium bromate titration for arsenic[6] gave a satisfactory end point, but too much time was required to heat the sample prior to titration, and the bromine fumes liberated were very annoying to the analyst when a large number of samples were analyzed daily.

In experiments conducted in 1937 it was found that no discoloration of the distillate and interference at the end point occurred if 10 ml. of 30 per cent hydrogen peroxide and sufficient water to moisten were added to 50 grams of soil in the distillation flask, the oxidation reaction was allowed to go to completion, and distillation was carried out in the usual

TABLE 1.—*Recovery obtained on laboratory-prepared samples of soil containing acid lead arsenate*

ACID LEAD ARSENATE ADDED TO SOIL—		SAMPLES ANALYZED	MEAN RECOVERY	STANDARD DEVIATION	RANGE OF RECOVERIES	
PER 3 KG. OF MIXTURE	PER 50 GRAM SAMPLE					
grams	*grams*		*gram*	*gram*	*gram*	*per cent*
9	0.15	45	0.1498	0.0008	0.1482–0.1519	98.8–100.3
12	0.20	50	0.1999	0.0009	0.1978–0.2015	98.8–100.7
15	0.25	25	0.2487	0.0015	0.2456–0.2530	98.1–101.2

manner. However, this peroxide treatment increases the time of a determination so much that it is impracticable when a large number of samples are being analyzed. Also, by conducting the distillation so that not more than 150 ml. was distilled in 2 hours, and making additions of

⁵ Greaves, J. E., *J. Am. Chem. Soc.*, **35**, 150–156 (1913).
⁶ *Methods of Analysis*, A.O.A.C., 1935, 41–43.

hydrochloric acid before the volume in the distillation flask was less than 75 ml., the discoloration of the distillate was reduced sufficiently to prevent obscuring of the end point.

In testing the method, three samples were prepared in the laboratory by thoroughly mixing 9, 12, and 15 grams of acid lead arsenate with enough air-dry soil to give 3 kg. of mixture; 50 gram samples were analyzed, and suitable aliquots were titrated with iodine of a known titer. The results were calculated as grams of acid lead arsenate per 50 grams of mixture. The results are shown in Table 1.

Another series of 20 samples was prepared by weighing 0.2000–0.2036 gram of acid lead arsenate directly into the distilling flask and adding 50 grams of air-dry soil. The recoveries ranged from 97.7 to 100.8 per cent of the added acid lead arsenate.

In the first year (1930) the distillation procedure was used, 746 samples of soil from nursery sections treated with acid lead arsenate were analyzed for arsenic. It was necessary to reanalyze about 3 per cent because duplicates did not check within 50 pounds of acid lead arsenate per 3-inch acre. After the technic had been mastered, practically no analyses had to be repeated.

METHOD OF ANALYSIS

Directions for sampling.—To obtain a representative sample in areas of 20,000 square feet or less, make, preferably, a composite of 50 borings.

Preparation of samples.—Take samples directly from the composite, air-dry, and store until analyzed.

Reagents.—Use the reagents described in *Methods of Analysis*, A.O.A.C., 1935, 41, 43, 3, 6.

Apparatus.—Use the apparatus shown in 4 of the same chapter of the A.O.A.C. methods cited above, with the following exceptions: (a) Use a spiral instead of a straight condenser, and (b) bend the delivery tube of the distillation flask so that when it is in position and connected to the condenser the condenser remains vertical. (This is done primarily to save space.)

Determination.—Place in the distilling flask 50 grams of air-dry soil containing arsenic. Add through the separatory funnel 50 ml. of the $N_2H_4 \cdot H_2SO_4 - NaBr$ solution and 50 ml. of HCl, close the flask, and heat very gently to boiling. Take considerable care at the beginning of the distillation to prevent the contents of the flask from boiling over. After the distillate has begun to pass over, increase the heat and conduct the distillation so that approximately 150 ml. passes over in 2 hours. When the volume in the flask is reduced to about 75 ml., add through the funnel 50 ml. more HCl and continue the distillation, repeating the addition of portions of HCl whenever the volume in the distilling flask is reduced to 75 ml. until 150–200 ml. of distillate has passed over. Wash down the condenser and all connecting tubes. Carefully transfer the distillate and washings to a liter volumetric flask, dilute to the mark, and mix thoroughly. Titrate the distillate with iodine as directed in 5(a), p. 42, of the A.O.A.C. methods. Calculate the results as pounds of acid Pb arsenate per 3-inch acre and round to the nearest 50 pounds.

SUMMARY

The distillation procedure specifying hydrazine sulfate-sodium bromide solution as the reducing agent, followed by an iodine titration, is a satis-

factory method for determining arsenic in soils treated with acid lead arsenate. The distillation is conducted so that approximately 150 ml. is distilled in 2 hours, and additions of hydrochloric acid are made before the volume is reduced to 75 ml. This reduces the color of the distillate so that the end point is not obscured. The appearance of color can be prevented entirely by oxidizing the organic matter in the distillation flask with 30 per cent hydrogen peroxide prior to distillation.

VOLATILE OIL IN SAGE

By J. F. CLEVENGER (U. S. Food and Drug Administration,
New York, N. Y.)

Most of the sage leaves used are grown in Jugoslavia and imported from Trieste. These are usually referred to as "Dalmatian" sage (*Salvia officinalis*). Limited amounts of sage herb are grown in Greece and imported from Athens. This is another species of sage, and it is usually referred to as Greek sage (*Salvia triloba*).

DALMATIAN SAGE

YIELD V/W*	SP. GR. 25°/25° C.	OP. ROT. 25° C.	REF. IND. 20° C.	AC. NO.	EST. NO.
1.9	0.925	+ 5.0	1.467	1.57	28.74
1.6	0.921	+ 5.2	1.465	2.23	20.71
1.6	0.923	+12.1	1.466	1.50	16.60
1.6	0.923	+13.5	1.463	1.50	19.12
1.8	0.923	+12.8	1.466	2.58	14.24
1.44	0.922	+ 4.5	1.462	2.2	21.30
2.3	0.917	+ 3.8	1.461	1.1	13.2
2.1	0.928	+15.2	1.464	3.5	21.5
1.7	0.937	+ 8.6	1.469	2.1	14.0
1.5	0.931	+ 7.9	1.464	0.9	10.8
1.2	0.935	+11.4	1.466	1.8	16.0

GREEK SAGE

YIELD V/W*	SP. GR. 25°/25° C.	OP. ROT. 25° C.	REF. IND. 20° C.	AC. NO.	EST. NO.
2.4	0.917	−21.0	1.469	1.2	
2.6	0.913	− 4.1	1.464	1.0	20.0
2.5	0.917	− 6.2	1.473	1.5	19.7
2.5	0.915	−17.3	1.468	0.57	15.8
2.45	0.915	−16.3	1.469	0.5	14.6
2.3	0.918	−13.1	1.466	0.9	13.0
2.2	0.917	−11.4	1.470	1.4	13.0
2.4	0.909	−12.4	1.469	1.35	21.4
2.5	0.911	−12.4	1.469	0.98	14.7
2.4	0.907	−13.8	1.470	0.87	25.2
2.1	0.916	−16.6	1.471	1.75	24.1

* cc. per 100 grams of sage.

During the past seven years many of the importations of sage leaves in New York have been analyzed for the yield of volatile oil. Determinations have also been made of some of the physical and chemical characteristics of these oils.

The results reported here were obtained by the method outlined in *Methods of Analysis, A.O.A.C.*, 1935, 447–449.

To determine the extent of loss of volatile oil in sage leaves upon exposure in the laboratory, a portion of some coarsely ground uniformly mixed sage leaves was analyzed. The remaining portion of the sample was stored in the laboratory in an open shallow pan. Seven months later the remaining portion was analyzed. The results follow:

DATE	YIELD v./w.	SP. GR. 25°/25°C.	OP. ROT. 25°C.	REF. IND. 20°C.	AC. NO.	EST. NO.
1/25/38	1.6	0.923	+12.11	1.464	1.22	16.24
8/15/38	1.2	0.929	+13.15	1.461	1.23	14.6

CONCLUSIONS

(1) This investigation was limited to a study of the volatile oil in Dalmatian and Greek sage. The volatile oil from Dalmatian sage always gave a positive rotation and that from Greek sage always gave a negative rotation. This characteristic would be of value in distinguishing between the two varieties studied.

(2) The yield of volatile oil is generally greater for the Greek sage than for the Dalmatian sage.

(3) The variations in the acid and ester numbers are not considered significant.

(4) After coarsely ground sage leaves had stood seven months in the laboratory in an open pan, it was found that a significant loss in volatile oil resulted. This determination indicates that on long exposure a material proportion of the volatile oil may be lost.

OBSERVATIONS ON THE COLORIMETRIC METHOD FOR VANILLIN*

By A. L. CURL and E. K. NELSON (Food Research Division, Bureau of Agricultural Chemistry and Engineering, Washington, D. C.)

In collaboration with the Federal Agricultural Experiment Station at Mayaguez, Puerto Rico, the junior author attempted to use the colorimetric method described in *Methods of Analysis, A.O.A.C.*, 1935, 307, in determining the vanillin content of Puerto Rico vanilla.

The results obtained by the colorimetric method were found to be much

higher than those by the gravimetric method, and a colorimetric determination made on the residue from the extraction of vanillin in the gravimetric method showed that a substance not vanillin remained and reacted with the reagent.

Accordingly, a study of the method was undertaken to find the cause of the discrepancy. The following table shows the results obtained when the lead reagent used to precipitate resins and tannins was a solution of 5 per cent neutral lead acetate and 5 per cent basic lead acetate for sugar analysis by the Horne method (later found to assay 68 per cent total lead and 16.8 per cent basic lead,[1] giving a ratio of neutral to basic lead in the final reagent of 6.3 to 1). Unless given otherwise, the vanillin results presented in this paper are expressed as grams per 100 cc.

	I GRAVIMETRIC	II COLORIMETRIC	III COLORIMETRIC AFTER EXTRACTION OF VANILLIN	IV SUM OF I AND III
10% Extract P.R. I	0.346	0.476	0.124	0.470
10% Extract P.R. II	0.206	0.336	0.134	0.340

In order to find whether this discrepancy in the results was due solely to the characteristics of Puerto Rico vanilla, 10 per cent extracts made from Bourbon, Java, Mexican, South American, and Tahiti vanilla beans* were examined. The following results were obtained:

	GRAVIMETRIC	COLORIMETRIC	DEVIATION
Bourbon	0.260	0.360	+0.100
Java	0.292	0.412	+0.120
Mexican	0.160	0.240	+0.080
South American	0.224	0.367	+0.143
Tahiti	0.168	0.218	+0.050

As it was then suspected that the basic lead acetate used was not sufficiently basic to precipitate all the interfering substances, a lead reagent was prepared with 10 per cent of the basic lead acetate containing 16.8 per cent basic lead (ratio of neutral to basic 3:1), No neutral lead acetate was added. The results are as follows:

	COLORIMETRIC	GRAVIMETRIC	DEVIATION
P.R.I.	0.356	0.346	+0.010
Bourbon	0.249	0.260	−0.011
Java	0.278	0.292	−0.014
Mexican	0.178	0.160	+0.018
South American	0.235	0.224	+0.011
Tahiti	0.159	0.168	−0.009

[1] Rosin, Reagent Chemicals and Standards, p. 224. D. Van Nostrand & Co. (1937).
* Specially made by David Michael & Co., Front & Master Sts., Philadelphia.

Then there was obtained a sample of basic lead acetate that assayed 73.4 per cent total lead and 33.5 per cent basic lead, a ratio of neutral to basic of 1.2 to 1. Using this sample the senior author made a lead reagent with 5 per cent each of neutral and basic, giving a ratio of neutral to basic lead of 2.8 to 1. This was used in the colorimetric determination on two extracts. The results follow:

	COLORIMETRIC	GRAVIMETRIC	DEVIATION
Mexican	0.171	0.160	+0.011
Tahiti	0.160	0.168	−0.008

To determine the effect of using a higher concentration of basic lead acetate, a 10 per cent solution with a ratio of neutral to basic lead of 1.2 to 1 was substituted for the 5 per cent neutral and 5 per cent basic. The results follow:

	COLORIMETRIC	GRAVIMETRIC	DEVIATION
Mexican	0.119	0.160	−0.041
Tahiti	0.104	0.168	−0.064

As described in *Methods of Analysis, A.O.A.C.*, 1935, and by Folin and Denis,[1] Leach,[2] or Snell,[3] the method gives no specifications for the basic lead acetate to be used, but these experiments show that the ratio of neutral to basic lead acetate should not be much greater or less than 3 to 1. A ratio of 6.3 to 1 was unsatisfactory, especially in vanillas of higher resin content, as it failed to precipitate interfering substances and caused high results. On the other hand, a reagent with a ratio of neutral to basic lead of 1.2 to 1 (10 per cent solution) carried down vanillin and led to low results.

The basic lead acetate used in making up the reagent for the colorimetric method for vanillin should therefore be assayed, and if the ratio of neutral to basic lead is much greater than 1 to 1 a new supply should be obtained.

The preservation of the standard vanillin solution used in the colorimetric method also requires attention. It was found that a standard vanillin solution increases in apparent strength when allowed to stand in a partially filled bottle. This is probably due to oxidation, as a full bottle, kept in the refrigerator, checked with a freshly made solution. A partially filled bottle kept several months at room temperature gave a color increase of 19 per cent over a freshly made standard.

[1] *J. Ind. Eng. Chem.*, 4, 671 (1912).
[2] Food Inspection and Analysis, 4th Ed., 1920, p. 922.
[3] Colorimetric Methods of Analysis, 2, 86–7 (1937).

In a colorimetric test on a solution of 0.1 gram of vanillic acid to the liter, 13 mm. depth matched a 0.1 gram/liter vanillin of 20 mm. depth, the vanillic acid giving about 50 per cent more color than the vanillin.

Folin and Denis[1] state that with the colorimetric method, with the standard set at 20 mm., no readings should be accepted as final if they fall much outside the limits of 15–30 mm.

In colorimetric determinations it is sometimes necessary to make a 1:2 dilution to bring the readings within the 15–30 mm. limit as shown in the following results:

	1ST READING	VANILLIN	2ND READING	VANILLIN	DEVIATION OF 1ST
					per cent
P.R. I	9.2	0.435	16.8	0.476	8.6
P.R. II	12.1	0.331	23.8	0.336	1.5

Snell[4] specifies a 20 per cent solution of sodium carbonate in place of the saturated solution used in the colorimetric method. This was found by the writers to be more satisfactory, as it causes less trouble with precipitation and is more convenient to prepare.

The solutions should be allowed to stand long enough to effect precipitation before being filtered, as a turbidity in the colorimeter will completely vitiate the results. Fifteen or twenty minutes is usually sufficient.

In carrying out the gravimetric vanillin method on extracts of pure vanilla beans, the ammonia extraction (designed to separate vanillin from coumarin) was dispensed with. Petroleum ether extractions of the vanillin residues were made, and the insoluble impurities, amounting to 3 to 14 mg., deducted from the weight of the crude vanillin.

The following table shows the effect of omitting the ammonia extraction in the analysis of authentic extracts of vanilla beans:

EXTRACT	NH_4OH EXTRACTION OMITTED	NH_4OH EXTRACTION PERFORMED	DEVIATION
Java	0.284	0.272	−0.012
South American	0.228	0.224	−0.004
Puerto Rico	0.332	0.340	+0.008
Bourbon	0.252	0.244	−0.008
Tahiti	0.152	0.147	−0.005
Mexican	0.156	0.147	−0.009
Average	0.234	0.229	−0.005

SUMMARY

(1) The basicity of the lead reagent should be high enough to equal a ratio of neutral to basic lead of approximately 3 to 1.

(2) The vanillin standard should be freshly prepared or kept in full bottles in a refrigerator. Old standards that have stood around in partly filled bottles should be discarded.

(3) To develop the color in the colorimetric method a 20 per cent solution of sodium carbonate is preferable to a saturated solution.

IDENTIFICATION OF FLAVORING CONSTITUENTS OF COMMERCIAL FLAVORS

VIII. SEMI-MICRO DETERMINATION OF THE AMIDO NITROGEN ATOM IN SEMICARBAZONES

By John B. Wilson*

While engaged in the work of identifying the volatile flavoring ingredients of a commercial flavor the writer encountered an aldehyde of unknown composition. A semicarbazone was formed, but the properties did not coincide with those of any known substance, and the quantity of material available was insufficient for the determination of nitrogen by Veibel's method,[1] which was used in Part I of this series[2] to establish the composition of the semicarbazones of several aldehydes and ketones. It seemed advisable to adapt the procedure to semi-micro proportions to permit such determinations to be made when 0.1-0.2 gram of sample is not available for the purpose, as required in the original procedure.

After some experimentation the procedure given below was worked out and found applicable to 10-25 mg. of sample, depending upon the content of nitrogen.

SEMI-MICRO DETERMINATION OF THE AMIDO NITROGEN ATOM IN SEMICARBAZONES

APPARATUS

Micro-Kjeldahl digestor.—Designed by E. P. Clark.[3]
Micro-Kjeldahl distilling apparatus.—Designed by E. P. Clark.[3]

REAGENTS

(a) *Potassium iodate solution.*—Dissolve 5 grams of KIO_3 in water and dilute to 100 cc.

(b) *Standard nitrogen solution.*—Place a quantity of NH_3 equivalent to about 40 mg. of N in a 100 cc. volumetric flask, add 25 cc. of water and a few drops of methyl red indicator, titrate with 0.1 N acid, and dilute to the mark with water (1 cc. of $N/10$ acid = 1.4 mg. of nitrogen in the standard solution.)

(c) *Sodium hydroxide solution.*—Dissolve 400 grams of NaOH in water and dilute to 1 liter.

(d) *Standard alkali solution.*—Use $N/70$ solution of NaOH, KOH, or $Ba(OH)_2$ (1 cc. of $N/70$ solution = 0.2 mg. of nitrogen).

[1] *Bull. soc. chim.*, 4th ser., 41, 1410 (1937).
[2] Wilson and Keenan, *This Journal*, 13, 389 (1933).
[3] *This Journal*, 16, 255 (1933).

DETERMINATION

On a tared piece of cigarette paper about 35×40 mm., weigh 10–25 mg. of sample expected to contain 1–2 mg. of nitrogen. Place paper and sample in a micro-Kjeldahl flask, add 5 cc. of H_2SO_4 (1+4), and heat just to boiling on the digestor for 15–25 minutes, or until the sample is completely hydrolyzed. During hydrolysis, note the odor of the aldehyde or ketone as a key to its identity. If the mixture begins to darken (due to charring) dilute with 1–2 cc. of water and continue the hydrolysis.

When the hydrolysis is complete, wash down the neck of the flask with 1–2 cc. of water, using a dropper; add 1 cc. of the KIO_3 solution, mix, return to the digestor, and boil until the iodine vapor is completely removed (about 10 minutes). Wash down the neck of the flask as before and cool.

Set the resistance connected to the steam generator of the micro-Kjeldahl distilling apparatus so that 10 cc. will distil in 6–7 minutes. Transfer the solution from the digestion flask to the distilling apparatus, washing the flask 4–5 times with small quantities of water (about 10 cc. in all). Seal the outlet of the condenser with a 125 cc. Erlenmeyer flask containing 10 cc. of ±0.02 N acid and 0.1 cc. of methyl red indicator. Add 5 cc. of the NaOH solution to the distilling flask and steam distil for 4–5 minutes. Lower the receiving flask so that the outlet of the condenser no longer dips below the surface of the acid solution and continue the distillation for 1.5–2 minutes to wash out the condenser tube.

When the distillation is complete, heat the receiver on a hot plate just to boiling for 1 minute. Titrate the excess acid while hot with standard alkali solution. Just before the end point is reached, boil again for 1 minute and finish the titration with 1 or 2 drops of alkali. If more alkali is needed, return to the hot plate for further heating until not more than 2 drops are required to make the color change. Find the titer of 10 cc. of acid solution in the same way as for the distillate from the sample and subtract the latter to obtain the acid equivalent to the nitrogen present.

To standardize the alkali solution, pipet 5 cc. of the standard nitrogen solution into the distilling flask, add 10 cc. of water and 5 cc. of the NaOH solution, and distil as with the sample into 10 cc. of the same ±0.02 N acid and 0.1 cc. methyl red indicator.

The procedure was applied to several semicarbazones with the results given in Table 1.

The data show about the same degree of accuracy for the semi-micro method as for the macro method, but the tendency is toward high results rather than toward low results as in the case of the work reported in Part I of this series. Results obtained by the method may be used in the calculation of the molecular weight semicarbazones by the formula given in that paper as follows:

$$\text{Mol. wt.} = \frac{1400.8}{\text{percentage of nitrogen found}} - 57.05.$$

SUMMARY

Veibel's method for determining one atom of nitrogen in semicarbazones has been adapted to a semi-micro scale.

Results obtained on several semicarbazones are of the same order as those obtained by the macro method.

TABLE 1.—*Nitrogen in semicarbazones by the semi-micro method*

SEMICARBAZONE	SAMPLE	NITROGEN FOUND		THEORY
	mg.	*mg.*	*per cent*	*per cent*
Acetone	11.2	1.37	12.23	12.17
	16.0	1.96	12.25	
Benzaldehyde	10.2	0.88	8.62	8.59
	15.7	1.38	8.79	
	19.2	1.68	8.75	
	20.0	1.74	8.70	
Heliotropin	16.5	1.11	6.72	6.76
	25.4	1.70	6.69	
	21.5	1.47	6.84	
p-Methyl acetophenone	13.8	1.01	7.32	7.32
	20.6	1.52	7.38	
Vanillin	21.6	1.45	6.71	6.70
	24.8	1.65	6.65	

IDENTIFICATION OF FLAVORING CONSTITUENTS OF COMMERCIAL FLAVORS

IX. DETERMINATION OF β-IONONE, WHEN 1–10 MG. IS PRESENT

By JOHN B. WILSON*

In Part V[1] of this series the writer presented a method for the quantitative determination of β-ionone and the results of testing it out upon quantities as low as 10 mg. The procedure has now been successfully applied to commercial flavors containing quantities of β-ionone ranging from 50 to 200 mg. per liter of sample.

With products containing less than 10 mg. of β-ionone it was found that slight variations in the procedure simplified the technic without affecting the accuracy of the method. As precipitation of β-ionone-*m*-nitrobenzhydrazide does not occur readily in 10 cc. of alcohol (1+1) unless 20 mg. or more of the ionone is present, it is unnecessary to await the beginning of crystal formation before diluting the solution to an alcohol content of 30 per cent by volume. When 10 mg. or less is present, the hot solution of the ionone and the *m*-nitrobenzhydrazide reagent in 5 cc. of alcohol may be diluted at once with 10 cc. of hot water, acidified, and set aside for the beginning of crystallization. When this procedure was followed, quanti-

ties of β-ionone as low as 2 mg. showed crystal formation within 30 minutes after being removed from the heat.

As a further aid in maintaining the accuracy of the method when such small quantities are to be precipitated, a number of ½ inch sintered glass disks were made from Pyrex glass according to the directions given by Kirk et al.[2] The disks were sealed into Pyrex tubing, which was then cut and fire polished so that crucibles 3 cm. long were formed with the disk about 1.5 cm. from the top. The crucibles weigh from 5 to 7 grams each and have a capacity of about 3 cc. It was found that 1 mg. of material could easily be removed from the disk for microscopic identification.

To accomplish filtration by suction a small flask was made from a heavy walled 50 cc. Erlenmeyer flask by adding a side tube of 5 mm. o. d. tubing about 50 mm. in length, at a point about 50 mm. above and ending about 80 mm. above the bottom of the flask. The neck of the flask was sealed off and built up for about 35 mm. above the seal. A drainage tube 5 mm. o. d. was then sealed in and the built-up portion flared to fit the crucible. A small piece of rubber tubing 1–1.5 cm. long was used to adapt the crucible to the flask (see figure).

Several solutions of known β-ionone content were prepared from a stock
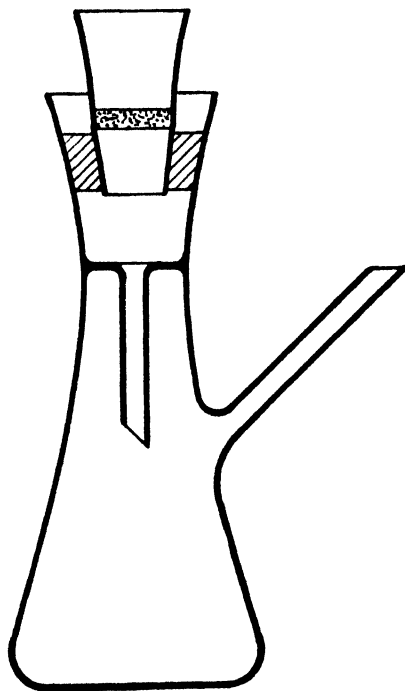
SUCTION FLASK USED WITH SINTERED GLASS CRUCIBLES

² *Ind. Eng. Chem. Anal. Ed.*, 6, 154 (1934).

solution, and the ionone was precipitated as β-ionone-*m*-nitrobenzhydrazide according to the following directions.

QUANTITATIVE PRECIPITATION OF β-IONONE WHEN 1-10 MG. IS PRESENT

Place 5 cc. of alcohol containing 1–10 mg. of β-ionone in a 50 cc. conical flask, add 90–95 mg. of solid *m*-nitrobenzhydrazide, and dissolve by warming the solution on the steam bath, taking precautions to prevent loss of alcohol through evaporation. Add 10 cc. of warm water, and if the solution becomes cloudy warm until clear. Remove the solution from the steam bath, add 0.2 cc. of glacial acetic acid, stopper the flask lightly, and place upon a wooden surface to prevent too rapid cooling. If 2 mg. or more of β-ionone is present, crystals begin to form withint 30 minutes after the contents of the flask have reached room temperature. Let stand in the room for at least 2 hours (overnight does no harm). Place in the refrigerator and leave overnight or up to 48 hours. Filter through one of the small sintered glass crucibles; wash with 15 cc. of cold 30% alcohol, using a wet policeman to remove precipitate adhering to the flask; and dry in a vacuum oven at 70° C. Weight of precipitate×0.541 = corresponding weight of β-ionone.

TABLE 1.—*β-ionone in varying dilutions of stock solution*

| SOLUTION | PRESENT | FOUND BY DETERMINATION | | | | RECOVERY |
		1	2	3	AV.	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*
A	7.7	7.4	7.2	7.0	7.2	92
B	5.8	5.4	5.1	5.1	5.2	90
C	3.8	3.4	3.4	3.5	3.4	89
D	1.8	1.8	1.9	1.5	1.7	90
E	0.96	0.8	lost	0.8	0.8	83

To establish the effect of the volumes of sample and distillate upon the recovery of β-ionone, a number of steam distillations of known quantities of β-ionone were made. Both the liquid in the distillation flask and the distillate collected varied in volume. These data are given in Table 2.

The distillations were carried out as described in Part V of this series except for the volume of distillate collected. The distillates were extracted with 60, 30, and 30 cc. portions of ether. Before the distillate was extracted the first portion of ether was poured through the condenser to obviate mechanical loss of β-ionone. The *m*-nitrobenzhydrazide (90–95 mg.) was placed in a 50 cc. conical flask, and the three ether extracts and 0.2 cc. of acetic acid were added and were evaporated in rotation. The residue was taken up in 5 cc. of alcohol, and the precipitation carried out as described previously.

The data in Table 2 show that while in some cases most of the β-ionone in a 250 cc. sample is contained in the first 100 cc. of distillate, 150 cc.

TABLE 2.— *Recovery of β-ionone from varying volumes of solution and distillate*

EXPERIMENT NUMBER	VOLUME OF—		β-IONONE	
	SOLUTION	DISTILLATE	PRESENT	FOUND
	cc.	*cc.*	*mg.*	*mg.*
1	250	150	8	8.2
2	250	100	8	7.4
3	250	50	8	6.8
4	250	100	8	7.0
5	250	100	8	6.7
6	250	100	8	6.4
7	250	100	10	8.7
8	250	100	10	8.4
9	250	100	10	9.8
10	250	150	8.7	8.5
11	250	150	8.7	8.4
12	500	150	8.7	8.2
13	750	150	8.7	8.0

should be collected in order to be sure of complete recovery. When the volume of sample is increased there is a gradual falling off in the recovery (11–12–13), again indicating the necessity of collecting larger quantities of distillate in the case of larger volumes of sample.

To test the applicability of the procedure to flavored food products, two 100 gram portions of a commercial strawberry flavored gelatin dessert were placed in liter flasks; 5 cc. of a 40 per cent alcohol solution containing 8.7 mg. of β-ionone was added to one and 5 cc. alcohol solutions containing 1.9 mg. of β-ionone was added to the other. Each sample was then dissolved in 250 cc. of water, and the determination of β-ionone was made as directed previously. The results are given in Table 3.

TABLE 3.—*Recovery of β-ionone from gelatin dessert solutions*

EXPERIMENT NO.	GELATIN DESSERT	WATER ADDED	DISTILLATE	PRECIPITATE	β-IONONE—	
					FOUND	PRESENT
	grams	*cc.*	*cc.*	*mg.*	*mg.*	*mg.*
1	100	250	150	16.1	8.7	8.7
2	100	250	150	3.2	1.7	1.9

The results reported in Table 3 show that the procedure recommended can be depended upon to give good recoveries of β-ionone when 1–10 mg. is present.

SUMMARY

The method for the quantitative determination of β-ionone given in Part V of this series was modified to permit its use for quantities of less than 10 mg. of β-ionone. The procedure gave good results when applied to solutions of known quantities of β-ionone even when interfering agents were present.

BOOK REVIEWS

The Chemical Formulary. A Collection of Valuable, Timely, Practical Commercial Formulae and Recipes for Making Thousands of Products in Many Fields of Industry. Volume IV. Editor-in-Chief, H. Bennett. Chemical Publishing Company of New York, Inc., New York, N. Y. 1939. 638 pp. Price $6.00.

Undoubtedly the majority of chemists are acquainted with the preceding volumes of this work. The current volume comprises 572 pages of new and additional formulae in the same fields included in the earlier volumes. It also contains a new introductory chapter for beginners in the art of compounding chemicals. The omission from this volume of the useful table of common names of chemical products is perhaps more t❸ be regretted than the further omission of the alcohol, temperature, and weights and measures tables.

To those who are not acquainted with this work, it may be stated that the sub-title of the book describes it very well. It is in fact more than the title indicates for in addition to formulae it has throughout its text concise but very informative explanations of the principles and practice of numerous useful arts. Its comprehensive character is indicated by the following titles picked at random from the index. "Artists' Crayons," "Retarding Staling of Bread," "Dog Bath Powder," "Vacuum Tube 'Getter'," "Destroying Yellow Jackets," "Worcestershire Sauce," "Stopcock Lubricants." The book is indeed an encyclopedia of chemical compounding.

The sections entitled "Beverages, Liquors and Flavors," "Cosmetics and Drugs," and "Food Products" should be of particular interest to members of the A.O.A.C. engaged in regulatory work. They contain clues to substances that are likely to be found in new products which present to the chemist analytical problems in the enforcement of laws relating to these products. It is regrettable that there is no warning in this volume to prospective manufacturers in regard to the applicability of the Federal Food, Drug, and Cosmetic Act and similar laws to many of the products described.

Though its primary appeal is to those engaged in the chemical arts, The Chemical Formulary contains abundant material of interest and practical usefulness to the worker in any field of chemistry.—EDWARD O. HAENNI.

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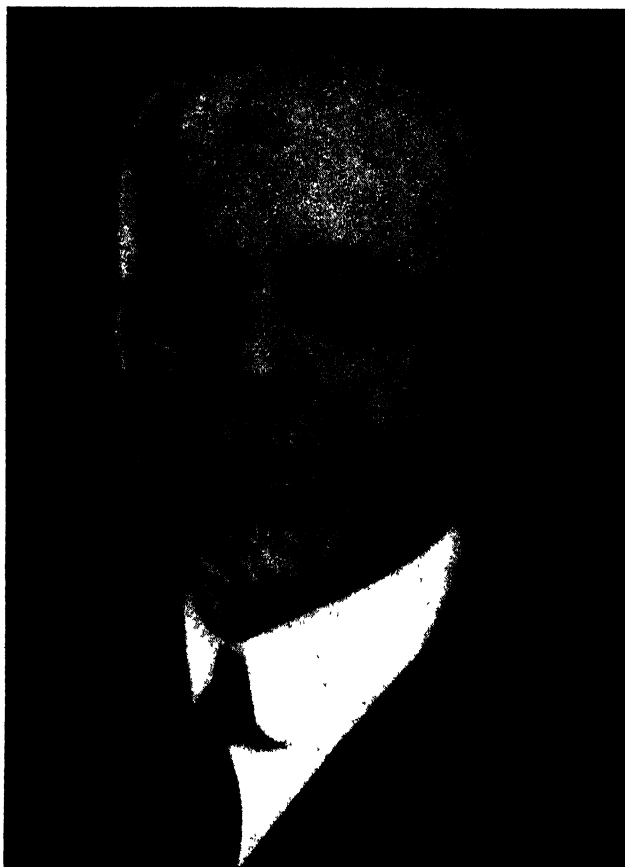
WILLARD DELL BIGELOW, 1866–1939

WILLARD DELL BIGELOW

Willard Dell Bigelow, twenty-fifth President of the Association of Official Agricultural Chemists, died on March 6, 1939. When this news was flashed through the press and scientific journals, not only his friends but all who knew his work in food chemistry and technology were deeply shocked. He was born at Gardner, Kansas, May 31, 1866. After completion of his High School studies, he attended Amherst College and was graduated in 1889. Then followed three years of graduate work and teaching at Oregon State College, Amherst, and Central High School, Washington.

On July 1, 1892, Dr. Bigelow entered the Bureau of Chemistry and began that long and brilliant service with Dr. Harvey W. Wiley in food chemistry, food technology, and food adulteration which culminated in the enactment of the Federal Food and Drugs Act of 1906. In the three years between 1904 and 1907 Dr. Bigelow was the author of no less than thirty bulletins and papers and the joint author of nine additional publications. This gives a partial picture of his almost ceaseless enterprise during this important period.

In 1913, Dr. Bigelow resigned as Assistant Chief of the Bureau of Chemistry to become director of the newly organized research laboratory of the National Canners Association. The work which he and his co-workers did stands out as pioneering in this specialized field of food technology, and it developed the fundamental scientific information on which practical technological operations could be predicated.

The first meeting of the Association of Official Agricultural Chemists that Dr. Bigelow attended was the tenth Annual Meeting, held in 1893. He served as a member of the Editing Committee in 1895 and of Committee C in 1904, as President in 1909 and as Editor and Secretary-Treasurer in 1912. He showed his interest in the work of this Association even after joining the National Canners Association, by attending all the annual meetings until the time of his death.

Dr. Bigelow had a personality that convinced a visitor at once of the strength of his convictions; he evidenced the true spirit of the scientist in that he knew his ground before he took a position or stand on any matter, and yet he was entirely free from bias and could clearly see the other angles or faces of any problem.

He was unusually willing to share with others the vast storehouse of knowledge gathered during his long experience in the fields of food chemistry, food technology, and food law enforcement. His unfailing kindness, his appreciation of the professional needs of less experienced men, and his readiness to offer practical suggestions on problems—all are attributes that stand out in the memory of those who were fortunate enough to have met and known him. One of his outstanding characteristics was his interest in the development and progress of younger men, to whom he was a source of stimulation and encouragement. The writer recalls many pleasant hours spent with Dr. Bigelow in which he pointed out with justifiable pride the progress made by many of the men who had been at one time or another associated with him.

Such a fine personality, such a keen grasp of the problems of technology, such a broad vision of science in relation to foods, their production and control, all combined in one man, make his loss to industry and to mankind truly a great one.

May his mantle of kindliness, ability, vision, and unselfishness fall on the shoulders of all of us who reaped the benefit of his wise experience, so that the words and deeds of Willard Dell Bigelow may guide us to lives of greater usefulness.

FRED C. BLANCK

TUESDAY—MORNING AND AFTERNOON SESSIONS—*Continued*

REPORT ON DRUGS

By L. E. WARREN (U. S. Food and Drug Administration,
Washington, D. C.), *Referee*

Last year 23 topics were assigned to associate referees—4 less than in the previous year. Substantial progress was reported in 21 of these subjects. From the reports of the several associate referees the Referee recommends for tentative adoption quantitative methods for 14 drugs. These are acetophenetidin, caffeine and acetylsalicylic acid in mixtures, emulsion of cod liver oil, guaiacol, mandelic acid, theobromine in theocalcin tablets, ointment of mercuric nitrate, sulfanilamide, hypophosphites, hexylresorcinol, chlorobutanol, and aspirin and phenolphthalein in mixtures.

In addition, microchemical tests for 7 substances were recommended for adoption as tentative by the associate referees. These are berberine, cotarnine, diallyl barbituric acid, narceine, mandelic acid, narcotine and sulfanilamide. The Referee recommends the tentative adoption of methods for each of these drugs.

The Referee recommends closure of 12 topics.

One subject (nitroglycerin in mixtures) was discontinued without the adoption of a method. Ten topics, hypophosphites, hexylresorcinol, phenacetine, caffeine and aspirin, chlorobutanol, aspirin and phenolphthalein, emulsions of cod liver oil, citrine ointment, sulfanilamide, gums, and theobromine calcium tablets, were closed and methods tentatively adopted for each. One method (for guaiacol) was adopted as tentative and the topic reassigned for further study of guaiacol in mixtures.

New French Pharmacopoeia.—Since the last meeting the new edition of the French Pharmacopoeia has appeared. It became official April 1, 1938. The last edition appeared in 1908, but several supplements had been published at intervals since that time. This book is published in two volumes. The smaller (660 pp.) contains the French laws affecting pharmacy, the standard solutions, and general analytical reactions; the larger (1200 pp.) contains the usual information given in the text of pharmacopoeias but it is much more encyclopedic, i.e. has more of what would be expected in this country in the dispensatories. Among the new features are colored plates of many drug plants. About 1300 drugs are described, whereas the U.S.P. describes only 568.

New Book on Drug Analysis.—A review of Dr. Garrat's book, *Drugs and Galenicals: Their Quantitative Analysis*, was published in *This Journal*,

21, 517. This work is by an English author. It deals chiefly with B.P. and B.P.C. preparations; consequently the references are mostly to English authors and methods. By legal requirements drug analysts in this country must use the U.S.P. or the N.F. methods in the analysis of official drugs for legal investigations provided methods are supplied by these compendiums. However, since many B.P. and B.P.C. preparations have their analogues in the U.S.P. XI and N.F. VI, the methods which the author has found workable for the British products could be applied in most instances to such American preparations as are not provided with assays. Also they may be used as checks on the official methods.

Microchemical Tests for Alkaloids.—This topic has been under consideration for nearly a score of years and nearly all the important medicinal alkaloids have received attention. Altogether one or more tests have been adopted for each of 33 alkaloids. A few of these are of synthetic origin. This year berberine, cotarnine, narceine and narcotine were studied. Tests were developed for each by the associate referee and his collaborators.

The Referee concurs in all of the recommendations made by the associate referee.

Microchemical Tests for Synthetics.—Microchemical methods for the identification of synthetics were first studied by the A.O.A.C. in 1932. To date tests have been adopted for 19 synthetic drugs, a few of which are synthetic alkaloids. This year diallyl barbituric acid, mandelic acid and some of its salts, and sulfanilamide were studied. The Referee concurs in the recommendations of the associate referee and suggests that plasmochine also be studied.

Hypophosphites.—Last year the bromine oxidation method was studied. Commercial sirup of hypophosphites was used as test material but known samples were not assayed. This year the method was subjected to collaborative study. The standard solution of bromide-bromate used was not that of the U.S.P., N.F. VI, or the A.O.A.C. The results obtained are good. The associate referee recommends that the method be adopted as tentative. The Referee is of the opinion that an attempt should be made to make use of the A.O.A.C. standard solution instead of the more expensive solution recommended by Bruening, *J. Am. Pharm. Assoc.*, **25**, 19, (1936). This phase of the subject was later brought to the attention of the associate referee. He made tests with the A.O.A.C. solution and found that the results were identical with those obtained with the more expensive solution. He then recommended that the A.O.A.C. solution be used. The Referee concurs.

Daphnia Methods.—The associate referee has continued his studies and has applied his method to tests for Vitamin E, certain alleged aphrodisiac drugs, cannabis, and numerous other toxic substances. The Referee recommends that the topic be continued.

Determination of Hexylresorcinol in Olive Oil.—Last year a method was

worked out which gave good results in the hands of three collaborators. Because of the limited amount of collaborative work done the topic was continued. This year but one collaborator worked on the problem. A specimen that gave 99.4 per cent (average) of recovery last year yielded 96.37 recovery after being kept for a year. Pure hexylresorcinol yielded 97.6 per cent recovery and a freshly prepared specimen in olive oil gave 97.8 per cent of recovery. The associate referee recommends that the topic be continued. The Referee believes that sufficient work has been done to warrant adoption of the method as tentative and so recommends.

Nitroglycerin.—The associate referee worked faithfully, but the results this year were as disappointing as they have been for the past two years. The associate referee recommends that the subject be discontinued temporarily. The Referee concurs.

Guaiacol.—Last year a method in the literature, which depends on the determination of the alkoxyl group, was adapted to the determination of guaiacol by the associate referee, *This Journal,* **21,** 543. This year the method was subjected to collaborative tests on known materials. The results are good. The associate referee recommends that the alkoxyl method be adopted as tentative. The Referee concurs.

Biological Testing.—No report was received from the associate referee. The Referee recommends that the subject be continued.

Iodine Ointment.—This topic has been under investigation for several years. The associate referee first developed a method for the determination of total iodine. This was included in the U.S.P. XI so that it was not adopted by the Association. Last year a method for the determination of free iodine was adopted tentatively, but with the provision that it be not advanced to official status, presumably until the subject could be closed. This year the associate referee and his collaborators studied methods for the determination of organically combined iodine. A method was developed which gave good results in a collaborative way on freshly made ointment but which failed on old specimens.

The Referee concurs in the recommendations of the associate referee.

Separation of Acetphenetidin, Acetylsalicylic Acid, and Caffeine.—This topic has been under investigation for several years. This year the associate referee applied a modification of the method previously tried. The recoveries of the three medicinal agents were as close to theory as could be expected in such a difficult separation. The Referee concurs in the recommendations of the associate referee.

Gums.—For five years the associate referee has attempted to identify various gums by precipitation methods. He has now applied various reactions to the precipitates and has developed tests which collaborative work indicates to be effective. The Referee concurs in the recommendations of the associate referee.

Theobromine Calcium Tablets.—This topic has been studied for two

years. This year the tentative A.O.A.C. method, *Methods of Analysis*, 1935, 590, was compared with the Boie process, *C.A.*, **25**, 169 (1931). The results from a limited amount of collaborative work are good. The associate referee recommends the deletion of the present tentative method and the substitution therefor of the Boie procedure. The Referee recommends that the method studied by the associate referee and his collaborators this year be adopted as a tentative alternative method, and that the status of the present tentative method remain unchanged.

Chlorobutanol.—Last year the associate referee applied the distillation method (with subsequent conversion to chloride) to the determination of chlorobutanol in mixtures. The results were not entirely satisfactory. This year the work was continued, and the collaborative findings are satisfactory. The Referee concurs in the recommendations of the associate referee.

Aspirin and Phenolphthalein Mixtures.—This is the fourth year that this topic has been studied. The Hitchens method, *J. Am. Pharm. Assoc.*, **23**, 1084 (1934), modified, was subjected to collaborative study. The results are moderately good. The Referee concurs in the recommendation that the method be adopted as a tentative method, and recommends that the topic be closed.

Aminopyrine and Phenobarbital in Mixtures.—Last year the associate referee devised an empirical method for determining each of these substances in admixture with each other but no collaborative work was done. This year the method was subjected to collaborative study on mixtures of the two drugs without excipient. The results are moderately good. The Referee concurs in the recommendation of the associate referee.

Elixir of Terpin Hydrate and Codeine.—The Association has adopted a method for the assay of terpin hydrate in the elixir (without codeine). Last year the associate referee and his collaborators developed an empirical method for determining both the terpin hydrate and the alkaloid. The results are reasonably good for codeine but are not entirely satisfactory for terpin hydrate. This year essentially the same method was tried again. The results are still not completely satisfactory for the recovery of terpin hydrate. The associate referee is aware of the inherent faults of the method but recommends its tentative adoption in the absence of a more accurate procedure. The Referee concurs.

A member of the Association criticizes the proposed method because of the small sample taken—only about 3 cc. of 0.02 N acid being required to titrate the codeine. For the alkaloid he uses a 50 cc. sample, dilutes with 50 cc. of water, and applies the double shake-out procedure. A little terpin hydrate appears with the alkaloid but this does not interfere with the titration. About 15 cc. of 0.02 N acid is required.

Emulsions.—This is the second year that this topic has been studied. Last year the associate referee tried various methods of extracting the oil

from emulsion of cod liver oil, but no collaborative work was done. This year a method was developed which gave satisfactory collaborative tests. Although the number of collaborators was not large and the method was not tried on commercial products, the associate referee recommends that the method be adopted as a tentative procedure.

Ointment of Mercuric Nitrate (Citrine Ointment).—This is the second year of study for this product. The associate referee and his collaborators used a modification of the method employed last year, *This Journal*, 21, 579. Good results were obtained. The Referee concurs with the associate referee's recommendation that the method be adopted.

Rhubarb and Rhaponticum.—No work was done. Rhaponticum is refused entry into the United States, so it was necessary to obtain a special permit to allow entry of specimens for experimental purposes. The topic should be continued.

Theophylline Sodium Salicylate.—Last year methods were developed for determining theophylline and salicylic acid, but the results were not considered sufficiently consistent for adoption of the methods. This year but little work was done. The Referee recommends that the topic be continued.

Sulfanilamide.—The associate referee tried several methods, of which one was selected. The Referee concurs with the recommendations of the associate referee that the method be adopted as tentative.

Mandelic Acid.—This is a new topic. The associate referee devised two qualitative tests for the acid. Some of the collaborators have questioned whether these tests are sufficiently characteristic for adoption. The quantitative tests consisted of shaking out a diluted, acidified solution with a mixture of chloroform and ether (2+1) and titrating the residue after careful removal of the solvent. The results are good.

The Referee questions whether the qualitative tests are sufficiently specific, therefore he concurs only in the recommendation that the quantitative assay be adopted as tentative.

REVISION OF METHODS

During the summer the Referee sent a circular letter to each of the associate referees and to certain of the former associate referees, which read in part as follows:

Owing to the important changes in the by-laws of the constitution of the Association, adopted at the last meeting, *This Journal*, 21, 101, it becomes more important than formerly that studies be made early concerning any possible changes in tentative or official methods already adopted. Two years (two readings) are required before a tentative method may be made official, final action, and additional collaborative work is expected although not obligatory. The same conditions apply to the deletion or amendment of an official method. It would seem desirable, therefore, at this time to make an editorial survey of the methods for drugs in *Methods of Analysis* and of those adopted since the book was revised, in order to determine whether any changes should be advised.

Each associate referee who is responsible for one or more methods in the drug section of the A.O.A.C., which are not now official, final action, is requested to make a critical editorial study of such methods and to report to the next meeting. This report might include one or more of the following:

(1) A recommendation as to whether the method should be deleted, with reasons.

(2) A recommendation as to whether the method should be retained as tentative, with reasons.

(3) A statement as to whether the method should be advanced from its present status (tentative or official, first action) to the status next higher (official, first action, or official, final action).

(4) Such other recommendation as in the opinion of the associate referee should be made.

Chloroform in Mixtures.—This method was developed chiefly to determine small quantities of chloroform in cough sirups. It consists in distilling the chloroform from a neutral solution in presence of alcohol into concentrated alkali and determining the resultant chloride. The method was adopted in 1931, although it was known for some years before that.[1]

Several minor criticisms concerning the method have been received. One is that low results are obtained. Roberts and Murray,[2] who first described the method in detail, obtained about 98 per cent recovery in a number of trials. Another criticism is that bumping results when the full amount of calcium carbonate (1 g.) is used. Bromides, chlorides, and iodides may be present in cough sirups, and the calcium carbonate is added to prevent the liberation of volatile mineral acids. Doubtless the quantity of calcium carbonate could be reduced although Putt (*loc. cit.*) recommended this amount. The Referee is of the opinion that dilution with water before distillation to prevent precipitation of sugar will overcome the bumping. The addition of glass beads should accomplish the result. The several criticisms received were submitted to each of the former associate referees on the subject, but owing to resignations and reassignments three associate referees had worked on this topic before the method was finally adopted. These chemists recommended that the subject be studied again by the A.O.A.C.

Associate Referee Moraw recommended that the words, "citrate bottle," in paragraph 105 be replaced by the words, "pressure bottle fitted with a rubber gasket that will provide a tight seal." He also recommended that the following caution be inserted in an appropriate place:

Caution.—Do not cool the pressure bottle suddenly. It is best to allow it to cool in the water in which it was boiled.

Associate Referee Kunke recommended the deletion of one of the reagent alcoholic potassium hydroxide solutions, 104 (a), on the ground that the stronger one, 106 (a) is sufficient.

The Referee objected to the expression " . . . fitted with a rubber gas-

[1] Putt, *Am. Food J.*, 10, 467 (1915); Lyons, Analysis of Drugs, ed. 2, p. 309 (1920).
[2] *Am. J. Pharm.*, 101, 654 (1930).

ket that will provide a tight seal," on the ground that some pressure bottles are provided with ground-glass plates, which are held in position by suitable screws.

In view of the criticisms offered and also the recommendations of the earlier associate referees, the Referee requested the last associate referee to make some additional trials. He has done this and has submitted a special report. He and his collaborators find that the tentative method yields about 98 per cent of the chloroform added. He recommends several minor amendments in the method, and the Referee concurs in these recommendations.

Aloin.—This method has been in the tentative stage since 1932, and no adverse comments have been received. The associate referee recommends that the method be advanced to "official, first action." The Referee concurs.

Bismuth Compounds in Tablets.—The associate referee recommends that this tentative method, *Methods of Analysis, A.O.A.C.*, 1935, 592, be amended by inserting before the beginning of the present text the following paragraph:

Count and weigh a suitable number of tablets and ascertain their average weight. Pulverize the tablets and preserve the powder in a tightly stoppered bottle.

He also recommends that the method be retained in a tentative state for the present. The Referee concurs in both recommendations.

Camphor.—This method, which depends on polarimetry, has been official for some years, *Methods of Analysis, A.O.A.C.*, 1935, 560, 51. The associate referee recommends its deletion on the ground that synthetic camphor may not be determined by it. In view of the fact that the dinitrophenyl hydrazine method has not given entirely satisfactory results the Referee recommends that no changes be made in the status of the A.O.A.C. method at this time.

The Referee further recommends that the statement, "Not applicable to synthetic camphor," be inserted in parentheses between the title and the text of the article.

Cascara Sagrada.—The tentative method for cascara sagrada, *Methods of Analysis, A.O.A.C.*, 1935, 583, has been criticized in that it requires too much time of the analyst. It has also been questioned whether the results obtained in the assay represent therapeutic value. One member of the Association believes that the method should be deleted. The associate referee is of the opinion that further work should be done. In view of these criticisms and the fact that no further collaborative work has been carried out, the Referee recommends that no changes in status be made at this time.

Cinchophen in Presence of Salicylates.—A method for this determination was studied last year. It was essentially the method devised by Emery.[1]

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[1] *J. Am. Pharm. Assoc.*, **17**, 18 (1928).

After suitable collaborative work had been carried out with good results the method was adopted as tentative. No criticisms having been received, the associate referee now recommends that the tentative method for cinchophen in presence of salicylates be advanced to official, first action. The Referee concurs.

Cocaine.—Two methods for the determination of cocaine have been adopted as tentative, *Methods of Analysis, A.O.A.C.*, 576. In the first the alkaloid is released by sodium bicarbonate, the alkaloid removed by ether, and the residue titrated. In the other the alkaloid is removed by petroleum benzin and the titration completed as usual. Provision is made for checking by conversion to benzoic acid and determining that. The associate referee recommends the deletion of the first method (96) because of the lack of originality. He recommends further the advancement of the second method to "official, first action."

The Referee therefore recommends that Method I (Section 96) be deleted, first action, and that Method II be advanced to official, first action.

Dinitrophenol (or its Sodium Compound).—A method for the determination of dinitrophenol was adopted tentatively in 1936, *This Journal*, **20**, 82. No adverse criticisms having been received the associate referee recommends that the present tentative method be made official, first action. The Referee concurs.

Ether.—A method for the determination of ether was adopted as tentative in 1932, *Methods of Analysis, A.O.A.C.*, 1935, 584. The associate referee reports that no adverse criticisms have been received and he recommends that the method be adopted as official, first action. The Referee concurs.

Homatropine in Tablets.—A method for the assay of this preparation was adopted as tentative after two years' study by the associate referee and his collaborators. The method is the well known "double shake out" procedure. No criticisms of the method having been received, the associate referee recommends that the method be advanced to official, first action. The Referee concurs.

Microchemical Tests for Synthetics.—In accordance with the policy of the Association to reduce the number of reagents as much as possible the associate referee recommends:

(1) That the directions for preparing bromide-bromate solution, 180 (b) be deleted and the following statement substituted: "Prepare as directed under 26 (c)."

(2) That the directions for preparing magnesia mixture, *Ibid.* (h), be deleted and the expression, "Prepare as directed under II, 7 (c)," be substituted.

(3) That the directions for preparing gold chloride solution, *Ibid.* (i), be deleted and the expression, "Prepare as directed under 176 (j)," be substituted.

(4) That the directions for preparing Kraut's reagent, *Ibid.* (m), be deleted and the expression, "Prepare as directed under 176 (b)," be substituted.

The Referee concurs in each of these recommendations.

Iodoform and Iodoform Gauze.—Methods for the assay of these preparations were adopted as tentative in 1931, *This Journal*, 15, 85. The Referee reports that no adverse criticisms have been received. He recommends that the status be advanced to official, first action. The Referee concurs.

Ipecac and Opium Powder (Dover's Powder).—This preparation is described in the U.S.P. XI, but no assay is provided. Other preparations of ipecac and opium, such as the tincture and tablets, are on the market, and a method for the assay of morphine in these products was published in 1934. This method has recently been modified.[1] The test depends on the formation of nitroso-morphine with nitrites in acid solutions and colorimetric comparison of this in ammoniacal solution with knowns. It is recommended that assay methods for ipecac and opium powder be studied.

Methylthionine chloride (methylene blue).—A tentative method for the assay of methylene blue was adopted by the Association in 1923, *This Journal*, 7, 20 (1923). Later this became official, *Ibid.*, 10, 69 (1927). This is an iodometric titration in the presence of acetic acid. It provides also for the determination of the drug in oil mixtures, solutions, etc. The U.S.P. XI adopted an iodometric method also, but since this does not provide for the separation of the drug from mixtures, the A.O.A.C. method was retained.

Methods for the assay of methylene blue have been studied in a collaborative way by a subcommittee sponsored by the American Drug Manufacturers Association and the American Pharmaceutical Manufacturers Association. This subcommittee found that the perchlorate method originally devised by Deahl and Maurina gave satisfactory results in collaborative trials. Essentially the method consists in precipitating an aqueous solution of methylene blue with an aqueous solution of potassium perchlorate and weighing the dried compound. The method was claimed by the subcommittee to be superior to the U.S.P. method (and presumably A.O.A.C. method). This information was brought to the attention of the associate referee, who made a preliminary study of the subject. He rejected the perchlorate method in favor of the A.O.A.C. and the U.S.P. XI methods. In view of his brief report the Referee recommends that no action be taken at this time.

Monobromated Camphor.—Two methods have been adopted for the assay of this product, *Methods of Analysis, A.O.A.C.*, 1935, 561. Method I is official and the other method is tentative. One converts the organically combined chlorine to chloride by sodium amalgam and the other uses

[1] *Quart. J. Pharm. Pharmacol.*, 10*_*468 (1937).

alcoholic alkali for the purpose. The associate referee is of the opinion that the second of these methods might be deleted. The Referee is of the opinion that each should be retained in its present status and so recommends.

Barbital and Phenobarbital.—The associate referee recommends that the official method, *Methods of Analysis, A.O.A.C.*, 1935, 582, 112, be amended, first action, by inserting at the end of paragraph 112 the following expression: "Determine the melting point to check the purity of the residue." The Referee concurs.

The associate referee also recommends that the tentative alternative method for barbital and phenobarbital (applicable in presence of stearic acid), *Ibid.*, 113, 582, be advanced to official, first action. The Referee concurs.

Sabadilla.—Methods for the assay of this drug were studied in 1929, *This Journal*, 12, 305. At that time the associate referee recommended that the method subjected to collaborative study be adopted as tentative. This was not done on the ground, *Ibid.*, 77, that the method was essentially a U.S.P. X procedure. The associate referee now recommends that the method then proposed be adopted as tentative. He points out that no official compendium in the United States has a method for the assay of sabadilla. The Referee has studied the literature since 1929. Gsterner recommends a method which is considerably different from that proposed by the associate referee. In view of these facts the Referee recommends that the subject be reassigned for 1939.

Pilocarpine Hydrochloride.—This salt is described in N.F. VI, but no assay is provided. The associate referee reports that he has had no criticisms of the method except the "rather indefinite parenthetical statement in regard to the sharpness of the end point." In view of the fact that he makes no recommendation the Referee believes that the present status of the method should not be advanced.

Phenolsulfonates.—A method was adopted as tentative in 1933. The associate referee has reviewed the subject and recommends that this method be retained in its present status. The Referee concurs.

Potassium Thiocyanate vs. Ammonium Thiocyanate as Reagent in Microchemical Tests.—In several instances potassium thiocyanate is used as a microchemical reagent in the identification of alkaloids and synthetics. In others the ammonium salt is employed. Owing to the greater availability of the ammonium salt, studies were undertaken by each of the associate referees to ascertain whether the ammonium salt could be used interchangeably with the potassium salt in the microchemical tests already adopted. Each associate referee reported that the reactions took place equally well with the ammonium salt. Therefore it is recommended—

(1) That the words "potassium thiocyanate" in the 15th line of par. 180, p. 605, be changed to read "Ammonium Thiocyanate."

(2) That the reagents under "Neocinchophen" and "Pyridium" on p. 606 be changed from "potassium thiocyanate" to read "ammonium thiocyanate."

(3) That the reagent for ethylhydrocupreine, "potassium thiocyanate," *This Journal,* **20,** 81 (1937), be changed to read "ammonium thiocyanate."

Thymol.—The Referee concurs in the associate referee's recommendation that this method be advanced to official, final action.

Thymol in Antiseptics.—The Referee concurs in the associate referee's recommendation that this topic be advanced to official, first action.

Wagner's reagent.—This reagent is described three times in the section on drugs in *Methods of Analysis* and each formula is different. Furthermore, none is exactly the same as the original formula described by Wagner in 1863. However, one is a close approximation.

The associate referee recommends that the directions for preparing Wagner's reagent, 180 (d), be deleted and the statement, "Prepare as in 176 (c)," be substituted. He further recommends that the direction in 5, p. 543, be deleted and the expression, "Prepare as directed in 176 (c)," be substituted. The Referee concurs.

Morphine in Sirups.—No adverse comments have been received for this method but the associate referee made no recommendation concerning it. The Referee recommends that the status be advanced to official, first action.

NEW SUBJECTS

Ointment of Yellow Mercuric Oxide.—The U.S.P. XI describes the preparation of ointment of yellow mercuric oxide and provides an assay for the product. An alternative method is desired for checking purposes. It is recommended that an associate referee be appointed to study this topic.

Aspirin, Phenacetin, and Salol.—Tablets containing mixtures of these three ingredients are on the market. The A.O.A.C. has adopted no method for the separation of these constituents in mixtures. It is recommended that this subject be studied.

Arecoline hydrobromide.—This salt is being used by veterinarians. It is described in N.F. VI, but no assay is provided. It is recommended that arecoline hydrobromide be studied with particular reference to the assay of the tablets.

Benzedrine.—This synthetic has come into considerable use in the last two or three years, but the Association has adopted no tests for it. It is recommended that benzedrine sulfate be studied.

Hydroxyquinoline Sulfate.—Hydroxyquinoline sulfate is marketed in various mixtures. The Association has adopted a microchemical test for its identification but none for its determination. It is recommended that chemical methods for the determination of hydroxyquinoline sulfate be studied.

Physostigmine Salicylate.—Tablets of physostigmine salicylate are marketed for use in ophthalmology, but no assay has been adopted by the A.O.A.C. It is recommended that assay methods for physostigmine salicylate tablets be studied.

Plasmochine.—The synthetic known as plasmochine has come into considerable use as an antimalarial remedy, particularly in mixtures with quinine. No microchemical tests for its identification have been adopted by the A.O.A.C. It is recommended that microchemical tests for plasmochine be studied, and that it be assigned for study for chemical methods of assay.

Separation of Acetanilid and Salol.—A problem arose involving the separation of acetanilid and salol. The A.O.A.C. has adopted two methods for separating phenacetin and salol, one of which has been included in N.F. VI. These methods were devised by Emery, Spencer, and LeFebre, but no process for separating acetanilid and salol was reported.[1] Both of the A.O.A.C. methods for phenacetin and salol were applied by several members of the Association to a specimen of commercial tablets of acetanilid and salol. Neither method gave consistent results. Several attempts to separate known mixtures of acetanilid and salol by the same methods failed to give satisfactory results. The Referee has used the Salkover[2] method with satisfactory results.

It is recommended that an associate referee on acetanilid and salol be appointed.

REPORT ON MICROCHEMICAL TESTS FOR ALKALOIDS

By C. K. GLYCART (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Continuing the subject this year, the Associate Referee made tests for berberine, cotarnine, narceine, and narcotine. Narceine, an alkaloid of opium, is characterized by its unusual property to form crystals, which are blue in color with Wagner's reagent, zinc chlor-iodide, and other iodized reagents. Stephenson[3] states that platinic chloride is the second best test for narceine and that beautiful feathery rosettes are formed from the amorphous precipitates in all solutions.

Narcotine, also an alkaloid of opium, forms crystalline precipitates in rosettes of needles with the reagent potassium hydroxide. Practically the same results are obtained with potassium acetate, sodium carbonate, and sodium phosphate.

Cotarnine, a derivative of opium, is recognized in the National Formulary as Cotarnine Chloride and is commercially known as "Stypticin." It is a yellow powder. According to Amelink,[4] potassium ferrocyanide

[1] *J. Ind. Eng. Chem.*, **7**, 681 (1915).
[2] *Am. J. Pharm.*, **88**, 484 (1916).
[3] Microchemical Tests for Alkaloids, p. 58 (1921).
[4] Schema zur Microchemischen Identifikation von Alkaloiden (1934).

forms characteristic crystals with cotarnine when acidified with hydrochloric acid.

In the preliminary work last year, the tests for berberine were considered insufficient for identification. In the work this year, clusters of radiating needles developed slowly with mercuric chloride if an excess of the reagent was avoided; crystals were readily obtained by the hydrochloric acid reagent with a saturated solution of berberine hydrochloride.

Directions for the tests, control specimens consisting of berberine hydrochloride, cotarnine chloride (N.F.), narcotine sulfate, and narceine hydrochloride, also samples for identification marked Nos. 1, 2, 3, and 4, were sent to the collaborators. The material for the controls was considered sufficiently pure for the work.

The methods were published in *This Journal*, 22, 88 (1939).

COLLABORATORS RESULTS AND COMMENTS

All the collaborators reported the correct identifications, namely, No. 1, Narceine; No. 2, Narcotine; No. 3, Cotarnine; No. 4, Berberine.

They commented as follows:

John R. Matchett, Bureau of Narcotics, Washington.—
Berberine.—It was found that a 1:100 solution of berberine hydrochloride could only be prepared in hot water and that crystals separated on cooling. Upon adding a drop of the hydrochloric acid reagent to a 1:400 solution or 1:200 solution, very fine needles formed immediately, and the number increased gradually as the drop stood. The needles formed with hydrochloric acid appeared to be quite distinctive, but crystals of a definite complex compound would seem to be preferable for identification to crystals of the alkaloid hydrochloride itself.

The $HgCl_2$ reagent produced an amorphous precipitate in the 1:200 solution and the 1:400 solution, but no crystals were formed even after standing one-half hour.

Cotarnine.—With the platinic chloride reagent a 1:200 solution of cotarnine hydrochloride gave large yellow sheaves of long needles along with long, very slightly curved, colorless, or faintly yellow needles. With a 1:200 solution the $HgCl_2$ reagent gave a precipitate, amorphous at first, quickly going over to rapidly growing long colorless needles. With a 1:200 solution the $K_4Fe(CN)_6$ reagent used as directed gave crystals as described, forming very slowly at the edge of the drop. With a 1:50 solution similar crystals formed much more rapidly. Of the tests proposed for cotarnine, the platinic chloride reagent appears best. The others, however, appear to be satisfactory except that with the $K_4Fe(CN)_6$ reagent crystal formation is exceedingly slow, especially in the more dilute solution.

Narceine.—With a 1:200 solution the platinic chloride reagent produced crystals as described, which formed slowly. There was no preliminary amorphous precipitate. With a 1:200 solution Wagner's reagent produced a thick mat of interlaced needles and burrs, forming first on the edge of the drop. They were so dark as to appear black rather than blue.

Narcotine.—With a 1:200 solution the KOH reagent produced crystals as described.

Irwin S. Shupe, U. S. Food and Drug Adm., Kansas City.—Merck's Index gives the solubility of berberine hydrochloride as 1 to 400. A saturated solution was satisfactory for both $HgCl_2$ and HCl tests. The 1:200 concentration of cotarnine seems

to be the best for all three tests for this alkaloid. Cotarnine ferrocyanide is especially characteristic. The narceine hydrochloride was not sufficiently soluble to make a 1:200 solution unless a small excess of HCl was present. Both a saturated solution and a 1 to 200 with excess HCl were satisfactory. The rosettes of needles described for narcotine formed more readily if the test drop was stirred slightly. (10% NH₄OH appeared to give the test more readily than KOH.)

J. H. Cannon, U. S. Food and Drug Adm., St. Louis.—The test for narcotine with KOH is not so quickly obtained as are the others, but good crystals were obtained on long standing. I did get crystals with narceine that were really blue this time.

F. K. Ballard, U. S. Customs Laboratory, Chicago.—

Berberine.—With HgCl₂. One part of berberine hydrochloride did not dissolve completely in 100 parts of water. Solution was filtered and filtrate used for test. Results seemed somewhat better when this solution was diluted with an equal amount of water. With 5% HCl the results were as given in the method.

Cotarnine.—With H₂PtCl₆ 1:200. Results as given in method. With HgCl₂ long branching needles as stated in method were observed, but the color appeared to be pale yellow. Chains of rhombic plates and tree-like forms were also observed. With K₄Fe(CN)₆ acidified with 1 drop of 5% HCl, the results were as given in the method.

Narceine.—With H₂PtCl₆. One part of narceine hydrochloride did not dissolve completely in 100 parts of water. The solution was filtered and the filtrate was used for this and the following test. Results were as given in method. The color of the rosettes was yellow, and several minutes were required for formation of rosettes. With Wagner's reagent a saturated solution of narceine hydrochloride was used. The results were as stated in the method.

Narcotine.—With KOH 1:200 the results were as stated in the method.

W. J. Rice of the Eli Lilly and Company, Chemical Control Laboratories, Indianapolis.—All these tests responded satisfactorily although it will be noted that we also observed brown plate-like octagons among the burr-shaped crystals in the K₄Fe(CN)₆ test for cotarnine.

Charles C. Fulton, Internal Revenue Service, St. Paul.—

Narceine.—Zinc KI₂[1] is strongly recommended for the blue needles. If traces of alkaloidal impurities are present in the narceine it is generally quite impossible to obtain these crystals with Wagner's reagent, and even with pure narceine Wagner's reagent will not crystallize so readily as zinc KI. Note that this reagent is *not* Stephenson's "zinc chlor-iodide," for which he gave a formula containing "free" iodine, and therefore having the same defects, as a narceine reagent, as Wagner's reagent. With platinum chloride—crystallized readily in rosettes, rather dense and dark—the crystals seemed to be poorly formed prisms rather than feathery forms when examined under high power.

I should rate the blue needles with zinc KI as easily the best test. The narceine solution need not be any particular strength; crystals are obtainable readily down to about 1:1600.

The arseni-molybdic acid reagent is sensitive to about 1:3300, and a dilute solution is recommended for the test. I am inclined to consider it the second best test; H₂PtCl₆ might come in as the third best test. It is less sensitive.

Narcotine.—The only test I know of at present is the crystallization of the free base. Any basic reagent will do, narcotine being such a weak base that it is thrown out even by KC₂H₃O₂. Larger crystals, prisms, can be obtained by precipitating from distinctly acid solution by concentrated KC₂H₃O₂. I think NH₄OH, or Na₃PO₄, or the like, may give a little better crystallization and a more sensitive test than potassium hydroxide; however, the test is about the same.

[1] Charles C. Fulton, *Am. J. Pharm.*, 104, 244 (1932).

Cotarnine.—This alkaloid gives a large number of crystals. I would not attempt to say at present whether the three tests you give are better or as good as any others available; however, all three are good.

Berberine.—As Stephenson remarks, a variety of reagents give very similar crystals. The test with 5% HCl is no doubt highly characteristic.

SUMMARY

The alkaloids were identified correctly by all the collaborators. In regard to the test for berberine, by mercuric chloride, Matchett reported that no crystals were found even after standing one-half hour. In the test for cotarnine with potassium ferrocyanide, Rice reported that amber-brown plates were observed among the burr-shaped crystals. In the test for narcotine, Shupe and Fulton stated that ammonium hydroxide is more sensitive than potassium hydroxide. Fulton recommended zinc potassium iodide for the blue needles in preference to Wagner's reagent for narceine.

These tests were repeated by the Associate Referee. Mercuric chloride with a 1:400 solution of berberine produced an amorphous precipitate, as correctly stated by Matchett. With a 1:800 solution, however, needles were formed. The test is not sufficiently distinctive and further study should be made.

The tests were modified to include the observations made by the collaborators.

Material for tests for coniine and cytisine were not available.

This year, the Referee on Drugs suggested that stovaine be studied. This will be included next year.

RECOMMENDATIONS[1]

It is recommended—

(1) That the microchemical tests for cotarnine, narceine, and narcotine be made tentative.

(2) That the test for berberine by hydrochloric acid reagent be made tentative.

(3) That further tests for berberine be studied.

(4) That coniine, cytisine, stovaine, and phenacaine (holocaine) be studied.

REPORT ON MICROCHEMICAL METHODS FOR SYNTHETICS

By Irwin S. Shupe (U. S. Food and Drug Administration, Kansas City, Mo.), *Associate Referee*

This seventh report on the subject of Microchemical Methods for Synthetics describes a study of tests for mandelic acid, sulfanilamide (p-aminobenzenesulfonamide), and diallylbarbituric acid.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, **22**, 56 (1939).

A study of the microchemical reactions of mandelic acid and sulfanil-amide was made by James W. Mitchell and reported in his unpublished thesis.[1] He shows that for sulfanilamide the following reagents produce crystalline precipitates: platinic chloride, picric acid, picrolonic acid, chromic acid, bromine water, and sodium nitrite. He also obtained characteristic reactions by diazotization and coupling with phenol and by precipitation of free sulfanilamide from acid solutions with sodium carbonate and other alkaline salts. For mandelic acid he obtained crystalline precipitates with zinc sulfate, cadmium chloride, mercuric chloride, mercurous nitrate, stannous chloride, copper sulfate, lead nitrate, silver nitrate, and potassium ferrocyanide.

Characteristics of synthetics studied

SYNTHETIC	SOLVENT	CONCENTRATION OF SYNTHETIC	REAGENT	DESCRIPTION OF TESTS AND CRYSTALS
Diallyl-barbituric acid	Dry powder	—	Lead tri-ethanol-amine	Stir a small amount of the synthetic into a drop of the reagent. Rods singly and in clusters.
	Dry powder	—	Barium hy-droxide	Stir a small amount of the synthetic into a drop of the reagent. Rods singly and in groups.
Mandelic acid	Water	1–100	Lead acetate	Rosettes of thin curving plates.
	Water	1–100	Mercurous nitrate	Burr-shaped groups of needles.
Sulfanilamide	Dry powder	—	Benzaldehyde	Stir thoroughly a small amount of synthetic into a drop of regent. 4-sided plates.
	0.1 N HCl	Saturated solution	Sodium nitrite	Yellow needles.

Yakowitz, *This Journal*, 21, 351, described microchemical tests for sulf-anilamide with benzaldehyde and cinnamic aldehyde. Benzaldehyde and sodium nitrite were considered especially suitable as reagents for sulf-anilamide. Lead acetate and mercurous nitrate were chosen as the best reagents for mandelic acid.

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[1] Thesis in partial fulfillment of Degree of Bachelor of Science in Chemistry, June 1938. Philadelphia College of Pharmacy and Science.

According to Itallie and Steenhauer,[1] diallylbarbituric acid unlike most other barbiturates forms a sparingly soluble crystalline salt with barium hydroxide. It also forms a crystalline precipitate with a solution of lead acetate containing triethanolamine.

The synthetics used in these tests complied with the N.N.R. standards for identity for diallybarbituric acid,[2] and for sulfanilamide.[3] The mandelic acid used was the racemic form with a melting point of 118°–119° C. Directions for the tests, control specimens of the synthetics, and unknown samples for identification were sent to collaborators.

The unknown samples were: No. 1, diallylbarbituric acid; No. 2, sulfanilamide; No. 3, mandelic acid.

The method was published in *This Journal*, 22, 89 (1939).

RESULTS OF COLLABORATORS

All the collaborators reported the correct identifications, namely No. 1, diallylbarbituric acid; No. 2, sulfanilamide; and No. 3, mandelic acid. The collaborators commented as follows:

J. H. Cannon, U. S. Food and Drug Adm., Chicago.—I have no adverse comments to make regarding these tests. For diallylbarbituric acid, barium hydroxide seems somewhat better than lead triethanolamine in that crystal formation is more rapid and the crystals seem to grow to a larger size.

W. J. McCarthy, U. S. Food and Drug Adm., St. Louis.—By following your directions the identifications were checked without much difficulty.

M. L. Yakowitz, U. S. Food and Drug Adm., San Francisco.—All of the tests responded nicely except the barium hydroxide test for diallylbarbituric acid. I repeated the test several times but was unable to achieve satisfactory results.

H. R. Bond, U. S. Food and Drug. Adm., Chicago.—The reagents formed crystal structures easily identified through the controls.

DISCUSSION

The unknown samples were correctly identified by each of the collaborators. Difficulty in the test for diallylbarbituric acid with barium hydroxide may be due to using too small an amount of the powdered synthetic in the test drop. The tests described are considered satisfactory for the identification of diallylbarbituric acid, mandelic acid, and sulfanilamide.

RECOMMENDATIONS[4]

It is recommended—

(1) That the microchemical methods presented for diallylbarbituric acid, mandelic acid, and sulfanilamide be adopted as tentative.

(2) That other important synthetics be studied such as para-phenylenediamine and para-toluenediamine.

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[1] Microchemie, Emich Festschrift, 1930, 166.
[2] New and Non-Official Remedies, 1937, p. 103.
[3] J. Am. Med. Assoc., 109, 358 (1937).
[4] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 56 (1939).

(3) That the directions for preparing bromide-bromate solution, *Methods of Analysis*, A.O.A.C., 1935, **XXXIX**, 180 (b), be deleted, and the following statement substituted: "Prepare as directed under **XXXIX, 26, (c).**"

(4) That the directions for preparing magnesia mixture, *Ibid.*, (h), be deleted and the expression, "Prepare as directed under II, 7 (c)," be substituted.

(5) That the directions for preparing gold chloride solution, *Ibid.*, (i), be deleted and the expression, "Prepare as directed under 176 (j)," be substituted.

(6) That the directions for preparing Kraut's reagent, *Ibid.*, (m), be deleted and the expression, "Prepare as directed under 176 (b)," be substituted.

REPORT ON HYPOPHOSPHITES

By Henry R. Bond (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The quantitative determination of hypophosphites in sirup preparations by bromine oxidation was subjected to collaborative study this year, the slightly modified method of assay devised by C. F. Bruening[1] being used.

Because of the good results obtained last year in brief experimental assays, it was decided to use as material for collaborative work three sirups prescribed in the National Formulary VI, (1) Sirup Ammonium Hypophosphite, (2) Sirup Hypophosphites, and (3) Compound Sirup of Hypophosphites. A quantity of each was prepared from hypophosphites previously assayed by the methods outlined in the National Formulary VI for the simple salts.

A sample of each sirup was sent to each collaborator. The method submitted was published in *This Journal*, 22, 90.

The results obtained by the collaborators are shown in the table.

DISCUSSION

The results of collaborative study are in good agreement with the calculated theoretical, which was obtained from assay results by National Formulary VI methods with the individual hypophosphite constituents. Such agreement among results is a strong indication that the non-hypophosphite ingredients (sugar, glycerol, etc.) have little or no effect on the bromine reagent when present in the concentrations prescribed by the National Formulary.

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[1] *J. Am. Pharm. Assoc.*, 25, 19 (1936).

(a) *Effect of other ingredients on bromine reagent.*—For purpose of verification, determinations were made by the Associate Referee, who used

Collaborative results

COLLABORATORS[*]	SIRUPS	AMMONIUM HYPOPHOSPHITE g. H₃PO₂/100 cc.	HYPOPHOSPHITES g. H₃PO₂/100 cc.	COMPOUND HYPOPHOSPHITES g. H₃PO₂/100 cc.
W. F. Reindollar, Baltimore		2.80	5.01	5.40
		2.80	4.98	5.42
		2.82	5.04	5.47
I. S. Shupe, Kansas City		2.85	5.05	5.51
		2.85	5.05	5.51
Jonas Carol, Cincinnati		2.76	4.91	5.39
		2.76	4.91	5.41
		2.76	4.92	5.41
C. B. Stone, Cincinnati		2.77	4.93	5.42
		2.77	4.93	5.42
H. G. Underwood, Cincinnati		2.77	4.94	5.41
		2.77	4.94	5.42
M. L. Yakowitz, San Francisco		2.84	5.04	5.52
		2.83	5.06	5.52
N. E. Freeman, Atlanta		2.82	5.05	5.52
		2.94	5.08	5.58
C. A. Wood, New York		2.82	4.98	5.44
		2.82	4.98	5.44
			4.99	5.45
H. R. Bond, Chicago		2.80	5.00	5.39
		2.80	5.01	5.39
		2.81	5.01	5.40
Average		2.81	4.99	5.45
Calculated theoretical		2.79	5.01	5.41
Average per cent of theoretical		100.71	99.60	100.74

[*] W. F. Reindollar, Maryland State Dept. of Health; all others, U. S. Food and Drug Adm.

preparations composed of the proper proportions of all ingredients except the hypophosphites; also, the individual non-hypophosphite ingredients were assayed in solutions of the same concentration of ingredients as in the prepared sirups. Average results of assays follow:

SIRUP PREPARED AS PRESCRIBED BY N. F. VI BUT WITH HYPOPHOSPHITES OMITTED	EFFECT ON REAGENT (CALCULATED AS G. $H_3PO_2/100$ cc.)
Ammonium hypophosphite	0.042
Hypophosphites	0.042
Compound hypophosphites	0.071
Individual non-hypophosphite ingredients	
Sucrose	0.042
Glycerol	0.000
Sodium citrate	0.000
Quinine and strychnine	0.034

The results of these determinations indicate that the non-hypophosphite ingredients exercise a reducing action upon the bromine reagent productive of slightly higher assay results. If corrections are applied to the average of the collaborators' results for the reducing action of these ingredients, the percentage relationship between the corrected average results and the calculated theoretical hypophosphite content of the sirups assayed is—

<div align="center">

per cent

Ammonium hypophosphite   99.28

Hypophosphites   98.80

Compound hypophosphites   99.46

</div>

However, since each aliquot used in the assays is equivalent to only 1 cc. of the original sirups, the effect of the other ingredients on the bromine reagent is so small that it falls within the limits of experimental accuracy among collaborators.

(b) *Stability of sirups.*—The stability of the hypophosphite content of the prepared sirups is illustrated by the fact that results of assays made seven months after compounding were as close to the theoretical figure as were results obtained from assays of the freshly prepared sirups, an indication that no oxidation of the hypophosphites had occurred.

(c) *Scope of applicability of the method.*—The bromine oxidation method devised by Bruening may be used in the assay of the hypophosphite sirups designated as official by the National Formulary, and also in the assay of such non-official sirups as contain no reducing agent other than hypophosphites, and no material of a phenolic character with which the bromine might react in the manner of Koppeschaar's reagent.

(d) *Bromide-bromate reagent.*—Supplementary assays were made by the Associate Referee, who substituted for the prescribed 0.1 *N* bromine solution, the standard bromide-bromate solution prepared as directed in *Methods of Analysis, A.O.A.C.*, 1935, p. 551. On the basis of assay results, the A.O.A.C. solution (3 grams $KBrO_3$ and 12 grams of KBr per liter) may be substituted for the prescribed solution.

RECOMMENDATION[1]

It is recommended that the volumetric bromine oxidation method of Bruening, with the A.O.A.C. standard bromide-bromate solution, be adopted as tentative for the quantitative determination of total hypophosphites in official National Formulary sirup preparations and in non-official sirups in which the non-hypophosphite ingredients are qualitatively and quantitatively similar to the official sirups, and that the subject be closed.

REPORT ON DAPHNIA METHODS

By ARNO VIEHOEVER (Philadelphia College of Pharmacy and Science, Philadelphia, Pa.), *Associate Referee*

The use of daphnia as a biological reagent or testing tool has been considerably extended. Its use for the evaluation of cathartics has been recommended in previous reports of the Associate Referee on Rhubarb and Rhaponticum, published in *This Journal,* and it was also suggested in a paper published in another journal.[2] William Tinsley,[3] of the Laboratory of Pharmacology and Therapeutics, College of Medicine, Chicago, Ill., has used the daphnia successfully in the study of cathartic action. He adopted the Vie-tubes for observation and also designed a perfusion stage for observations on daphnia.[4]

N. Tischler and the writer[5] have used daphnia in the testing of insecticides (especially naphthylisothiocyanate), and H. Mack and the writer[6] have used it for the testing of toxic as well as laxative substances isolated from May apple root, *Podophyllum peltatum.*

Various other workers and laboratory organizations have become interested in experimenting with daphnia, e.g., Dr. Ned Proescher of Santa Clara County Hospital, San Jose, Calif.; Mr. Malin, Chemist of the Immunity Research Laboratories, Glendale, Calif.; Mr. Raybin of the Chemical Laboratory, New York City Health Department; and Dr. Fanto of the McKesson and Robbins Research Laboratories.

Testing of snake venoms and antivenins with daphnia has been carried out in the referee's laboratory with the assistance of Mr. Quimba, of digitalis standardization with Messrs. Sokoloff and Taransky; and research on other problems, indicated below, with Dr. Isadore Cohen.

The following assays are suggested for tentative methods:

APPROXIMATE ASSAYS FOR CANNABIS (MARIHUANA)[7]

Make a simple test for the narcotic principle in cannabis by extracting a small representative sample (e.g., 10 grams) for 30 seconds with a fairly stable reagent

[1] For report of Subcommittee B and action by the Association, see *This Journal,* 22 56, 90 (1939).
[2] *J. Am. Pharm. Assoc.,* 27, 668 (1938).
[3] *J. Lab. Clin. Medicine,* 23, 985–990 (1938).
[4] *Ibid.,* 1076.
[5] *Soap,* 14, 109–123 (1938).
[6] *J. Am. Pharm. Assoc.,* 27, 632 (1938).
[7] Arno Viehoever, *Am. J. Pharm.,* 109, 1 (1937)

(3 cm.) consisting of isopropyl alcohol, containing .05 per cent sodium hydroxide, and .05 gram of highly adsorbent charcoal (e.g., Nuchar 000) added with the cannabis.

A positive test, recognized by the practically immediate appearance, after filtering, of a color change from almost colorless to pinkish to deep red evidently indicates the presence and amount of cannabinol. The approximate amount apparently can be determined colorimetrically.

Spontaneous evaporation of this reddish liquid leaves a dry, partially pinkish to violet and rather persistent residue. This dissolves to an orange red solution in strong ammonia and with a violet to almost bluish violet color in acetone.

Biological Method.—Triturate the benzene-free residue of a benzene solution of 0.1 gram in about 2 cc. of culture water, containing possibly 0.1 per cent of acetone to speed solution of cannabinol.

The narcotic effect of cannabis extract becomes especially noticeable by comparing the swimming behavior of the daphnia in the culture water containing cannabis, with daphnia swimming in the cannabis-free culture medium, without or with acetone.

So-called locomographs, recorded in the comparoscope, indicate the lowered level of swimming, the reduction in speed and distance of swimming, as measured by the same time unit chosen under the influence of narcosis. So-called "shadowgraphs," obtained by the simultaneous photographing of daphia in 2 chambers, one with, the other without cannabis, permit the observation of the lowered level of swimming in the case of cannabis, causing a progressive debility, leading to death. The progressive speed and extent of narcotic debility evidently depend upon the concentration.

APPROXIMATE BIOLOGICAL ASSAY FOR VITAMIN E AND TOCOPHEROLS[1]

I. *Preparation of vitamin E. deficient media.*—Exhaust Wizard sheep manure (20-mesh powder) with petroleum ether. Use 1 gram of this extracted manure to 1 gallon of dechlorinated tap water for the preparation of the deficient culture medium.

II. *Transfer of daphnia magna.*—Transfer gravid females into this medium from a normal Wizard sheep manure culture. A medicine dropper with the tip cut off has served well for this purpose, as practically no liquid need be transferred with it, if the analyst traps the daphnia within the dropper, removes liquid inside and out with blotting filter paper, and inserts the dropper for the release of daphnia into the deficient medium.

III. *Breeding of vitamin E deficient daphnia.*—Provide the same cultural conditions as for normal standardized daphnia. Separate any young that might be born between the 10th and 20th day. From this deficient culture medium select 20-day-old daphnia and record ovarian development as follows: − none; + oocytes visible; + + bluish green colorative in ovaries, individual eggs roughly outlined, gravid; with number of embryos released in brood sac.

IV. *Restoration of deficiency.*—Place groups of 10 daphnia in separate museum jars, 4.8×10×15 cm., filled with 8 ounces of the same deficient culture medium, using one jar for control and at least two jars for the experiments with the test substance in amounts of 1 mg., or in quantities below or above that amount. Follow the general growth and the internal development carefully. Note and record especially whether the ovarian dysfunction is removed and the rapid reproductive cycle or rhythm is reestablished, grading the ovarian response as before.

V. *Comparative tests.*—For comparison observe the effect upon daphnia placed in culture medium with four drops of reasonably fresh wheatgerm oil (Tritricol)

[1] Viehoever and Cohen, *Am. J. Pharm.*, 110, 1 (1938).

added to a gallon of this vitamin E deficient medium,—resulting in the cure of ovarian dysfunction and the reestablishment of the reproductive rhythm.

VI. *Increased fecundity.*—Grow daphnia in groups of 10 in museum jars filled with 8 ounces of regular Wizard or Bovung medium under uniform conditions and determine the distribution curve for the number of young released in the initial clutches. Add the substance (mg. quantities) to groups of 1 day old daphnia, grow under like conditions, and determine their distribution curve for the number of young in the clutches. A significant increase strongly indicates vitamine E properties of the test substance.

BIOLOGICAL ASSAY FOR APHRODISIACS AND IRRITANTS[1]

I. *Breeding of male daphnia magna.*—Maintain crowded cultures of daphnia at sub-optimum nutritional conditions. Select mature vigorous males.

II. *Standard reference substances.*—Cantharidin in solution of culture water (1.0 gram: 30,000 cc.); or yohimbine in solution of culture water (0.1 gram: 100 cc.).

III. *Preparation of daphnia for the test.*—Evacuate the intestine with 1:100 dilution of alcohol-free fluidextract of cascara sagrada. Return daphnia to culture medium for 1 hour, then mount the daphnia in hanging drop preparations of solutions of cantharidin, of yohimbine, and of the test substance at known and comparable concentrations.

IV. *Observations made with compound microscope.*—

(1) Note effect on penis-like male sex organ—of both daphnia without and with evacuated intestine, observing increased movement of the excited sex organ and possibly the ejaculation of sperm.

(a) Compare reaction carefully with cantharidin, if ejaculation occurs.

(b) Compare with yohimbine, if only increased movement of the excited sex organ occurs.

(2) Record local effects upon the tissues and especially the mucosa of intestine, and general effects upon various organs and organ functions as intestine, liver, kidney, heart beat, respiratory rate, and eye movement.

(3) If tests show similarities (a) to cantharidin action, compare as in general toxicity procedures with cantharidin 1:55,000, as standard reference solution; (b) to yohimbine action, compare similarly with .1% yohimbine hydrochloride as the standard reference solution.

BIOLOGICAL ASSAY OF TOXIC SUBSTANCES[2]

I. *Standardization of daphnia.*—Maintain and standardize the cultures as reported by Viehoever, and Viehoever and Cohen. The use of daphnia grown in the same medium (at 68°–72°F. in northern light) is recommended for a series of comparative tests.

II. *Conditions of environment.*—Preferably conduct the tests under the same light, temperature, etc.

III. *Standard procedure.*—Fit museum jars, measuring 14½×9½×1½ cm. with 100 cc. of the test solution, made with the test solution used for the growing of test daphnia. Divide the jars by markings or lines on the outside into as many equal zones as are necessary for the preciseness of the test (a detail adjusted for the particular toxic agent in question). Use 50 daphnia, as the minimum, for every test.

IV. *Theory of test.*—The normal functioning of the swimming mechanism of the daphnia is so characteristic that any impairment to it can be considered the result of a toxic influence. Four general reactions are produced, depending on the nature

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[1] Viehoever and Cohen, *Am. J. Pharm.*, 110, 6 (1938).
[2] *Ibid.*, 109, 285 (1937).

of the agent: (1) Incoordination (impairment of normal swimming); (2) excitation, or even convulsions forcing the ascent of daphnia, followed by depression or paralysis, causing their descent; (3) progressive depression or paralysis; (4) precipitate depression forcing the more or less rapid descent.

All conditions being standard or constant, and the only variable being concentration, the following relationship holds true:

Debility of daphnia (inability to ascend) = concentration of test substance X time. The inability to ascend may be measured by arbitrary zones of locomotion. Count the number in zones at definite time intervals, and continue long enough to reach the end point at which all daphnia are down but not necessarily dead.

V. *Comparative tests.*—Examine the action of the standard reference agent upon the swimming mechanism of daphnia. Ten animals may be used for this purpose. If the reaction falls under IV, 1–3, proceed as follows: Establish the debility response for representative concentrations for 0.1, .01, .05, and .001%. The concentrations selected will be governed by the degree of toxicity of the agent. More dilute solutions tend to give more accurate results, although increasing the length of time for the necessary sequence of observations.

Check the test agents at concentrations where the standard reference causes a debility shift from 6–8 hours. Avoid higher concentrations since small differences in activity might not be readily observed. Compare carefully the swimming behavior in standard and test solutions.

VI. *Testing of single-active principles.*—Making additional observations where necessary, record the heart beat and respiratory rates of a representative number of daphnia at the start of the assay. Record the same at the end of the assay in standard reference and test solutions. This method of assay is suitable for single active principles, like strychnine, pilocarpine, picrotoxin, etc.

VII. (1) *Testing of U.S.P. tinctures and fluidextracts.*—If the products owe their activity to one important single substance, select this substance for use as the reference standard. Determine its action as suggested above. Determine the pH of the tincture or extract. If alkaline, add enough dilute tartaric acid to make it acid to litmus paper. Evaporate by fan at room temperature to highly viscous consistency. Dilute with distilled water to 5 times the original volume. Stir thoroughly and set aside for 15 minutes. Stir again and filter. Take 5 cc. of the filtrate as the equivalent of 1 cc. of tincture or fluidextract, whereas 5 cc. of filtrate + 95 cc. of culture water would equal 1–100 dilution of tincture or fluidextract. Proceed as indicated previously with a measured portion, using the same amount of the unfiltered mixture for a check.

(2) *Testing U.S.P. tinctures and fluidextracts.*—If they owe their activity to a chemical complex, select one lot as a reference standard and make relative comparisons, proceeding as in VIII. Grade on basis of fastest debility shift at the same concentration and in the same time.

VIII. *Testing of tinctures and fluidextracts (not U.S.P.).*—If they owe their activity to a chemical complex, prepare from a representative identified material a tincture or extract, according to U.S.P. method. Use this as a standard reference and proceed as previously directed.

Similar methods, with or without modification, may be used for testing such substances as veratrum[1] and marihuana, and for the evaluation of organic synthetics and their derivatives (e.g., amphetamine[2] etc.). Permanent records may be made with locomographs and shadowgraphs.

[1] *Am. J. Pharm.*, 111, 3 (1939).
[2] *Ibid.*, 110, 12 1938).

REPORT ON HEXYLRESORCINOL

By MORRIS L. YAKOWITZ (U. S. Food and Drug Administration,
San Francisco, Calif.), *Associate Referee*

At the last meeting of the Association, a method for determining hexyl-resorcinol in olive oil was presented. This method was used by the associate referee and two collaborators in assaying a solution of hexylresorcinol in olive oil, which was made up to contain 1.486 per cent by weight of hexylresorcinol. The results obtained by the analysis indicated that the method was satisfactory.

After this known mixture had aged for about one year, it was assayed by F. A. Rotondaro of the Philadelphia Station. He obtained a recovery of 1.43 per cent by weight of hexylresorcinol, which is equivalent to 96.3 per cent of the amount added. He also carried a weighed amount of hexyl-resorcinol without olive oil through the method and obtained a recovery of 97.6 per cent. He then made a solution of hexylresorcinol in olive oil that contained 2.84 per cent by weight of hexylresorcinol. Assay of this solution gave a recovery of 2.76 per cent and 2.80 per cent, corresponding to 97.2 per cent and 98.5 per cent of the added hexylresorcinol.

Rotondaro's work indicated that the use of hydrazine in the method may be unnecessary. It is therefore recommended[1] that the method be studied during the coming year with the objective of simplifying it.

REPORT ON ERGOT

By LLOYD C. MILLER (U. S. Food and Drug Administration,
Washington, D. C.), *Associate Referee*

The colorimetric method, which was used in last year's collaborative work, was studied further. This method has been used to determine the amount of alkaloids extracted from crude ergot by ether (Soxhlet) and by the U.S.P. menstruum (Process C percolation with acidified 50 per cent alcohol). The latter extraction process appears to remove 50–75 per cent of the amount of alkaloid that can be extracted with ether.

REPORT ON NITROGLYCERIN IN MIXTURES

By OMER C. KENWORTHY (U. S. Food and Drug Administration,
New York, N. Y.), *Associate Referee*

The method used last year appeared to be satisfactory, but when submitted to collaborators, unexplainably the results showed shortages in the neighborhood of 30 per cent.

This year an attempt was made to find out the reason for the shortages.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, **22**, 56 (1939).

Determinations were made by essentially the same method as last year, but certain procedures were modified. For example, the amount of acid was varied from 1 drop to 5 cc., and the time of shaking was varied from 5 minutes to 1 hour. Absolute alcohol instead of the ordinary 95 per cent alcohol was tried, and the acid was added both before and after filtering. All variations yielded a return of approximately 80–90 per cent of nitroglycerin.

When the amount of alcohol was increased to 100 cc. (50 cc. used previously) slightly higher results were obtained, but still they were approximately 10 per cent short. Attempts to extract the nitroglycerin with ether or amyl alcohol resulted in rather complete failures; an attempt to use alcohol neutralized to phenolphthalein gave a shortage of approximately 40 per cent.

A series of determinations was made with 50 cc. of alcohol. The liquid was centrifuged and filtered into a 200 cc. volumetric flask, and the process was repeated with 25 cc. of alcohol, until 200 cc. of filtrate was obtained. Nitroglycerin was run on a 100 cc. aliquot, by the method outlined last year, except that no acid was present in the alcohol, 2 cc. being added just before distilling. Six such determinations gave an average recovery of 95 per cent. An attempt to control the acidity of the powdered extracts by using quinine alkaloid resulted in a recovery of about 75 per cent.

Since a recovery of 95 per cent could be obtained by centrifuging and making to volume, this method, with a new supply of nitroglycerin, was submitted to collaborators. Due to the nearness of the annual meeting it was submitted before the associate referee had completed his work. The results of three analysts from the Food and Drug Administration who used the method follow:

	grain/tablet	
J. C. Molitor, New York City	0.0203	0.0206
E. H. Grant, Boston	0.0199	0.0194
O. C. Kenworthy	0.0195	0.0202

As the tablets contained 0.0236 grain/tablet (av. of 6 determinations) it can be seen that the shortages on the collaborative samples amount to 15–20 per cent.

Grant commented as follows: "There was still much green color in the last 25 cc. portion of alcohol, but theoretically there should be about 99.5 per cent extraction of the nitroglycerin."

This year as last year, a method that seemed promising failed to give good results when tried by collaborators. No method so far tried has resulted consistently in a good recovery of nitroglycerin. It is recommended[1] that the work be dropped temporarily.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 56 (1939).

REPORT ON GUAIACOL

By KENNETH L. MILSTEAD (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The Viebock and Schwappach method as modified by E. P. Clark for the determination of alkoxyl groups was applied to the determination of guaiacol and guaiacol derivatives last year. This year the method was subjected to collaborative investigation. Two samples were sent to collaborators.

Sample No. 1 consisted of guaiacol carbonate and conformed to N.F. VI purity tests.

Sample No. 2 consisted of synthetic guaiacol.

The method was published in *This Journal*, **22**, 91, 100.

Owing to the special apparatus required for this determination only a limited number of collaborators are available. Results were obtained from four on guaiacol carbonate and from two on guaiacol. The findings of the collaborators and the results obtained by the Associate Referee follow.

Collaborative Results

COLLABORATOR	SAMPLE NO. 1 GUAIACOL CARBONATE FOUND	SAMPLE NO. 2 GUAIACOL FOUND
M. Harris Chicago	99.8 99.9 99.8	
S. M. Stark, Jr. St. Louis	100.0 99.7	
E. P. Clark Washington	98.7 98.2	98.4 98.6
S. Reznek New York	100.5	99.3 100.0
Associate Referee	99.8 99.9 99.8	98.6 98.3

The determinations by Clark were made on samples weighing between 13 and 15 mg., and 0.05 N thiosulfate was used.

Comment of Collaborator S. Reznek.—I took the liberty of making the following modifications, which I have used in routine work with the apparatus:

(1) Use of 3 cc. of liquefied phenol (90% phenol—10% water), instead of 2.5 cc. of crystalline phenol, to avoid the necessity of melting the phenol.

(2) Use of a single 15 ml. test tube containing 10–12 cc. of bromine solution, instead of the two receivers "C" and "D."

It is recommended:[1] That the Viebock and Schwappach method as modified by E. P. Clark for the determination of alkoxyl groups be adopted as a tentative method for the determination of guaiacol and guaiacol carbonate.

No report on biological testing was given by the associate referee.

REPORT ON IODINE OINTMENT

By William F. Reindollar (State of Maryland Department of Health, Baltimore, Md.), *Associate Referee*

Iodine ointment is a suspension of a glycerol solution of iodine and potassium iodide in a base composed principally of petrolatum. When

Collaborative results on iodine ointment

COLLABORATOR	SAMPLE A				SAMPLE B			
	FREE IODINE	AV.	ORGANICALLY COM-BINED IODINE	AV.	FREE IODINE	AV.	ORGANICALLY COM-BINED IODINE	AV.
H. R. Bond	3.27		0.98		3.66		0.07	
	3.25	3.26	0.93	0.95	3.64	3.65	0.07	0.07
W. D. Dembeck			0.70				0.06	
			0.78				0.06	0.06
			0.82					
			0.85	0.79				
H. E. Chaney	3.32		0.33		3.65		0.08	
	3.29	3.31	0.45	0.39	3.64	3.65	0.07	0.08
J. C. Jones	3.24		0.66		3.47		0.07	
	3.22		0.72	0.69	3.51		0.06	0.07
	3.47				3.51			
	3.46	3.35			3.49	3.50		
H. J. Fisher	3.35		0.39		3.64		0.11	
	3.40	3.38	0.40		3.67	3.66	0.12	0.12
			0.48	0.42				
J. W. Todd	3.29	3.29	0.17	0.17	3.50	3.50	0.17	0.17
L. T. Ryan	3.40		0.56		3.53		0.10	
	3.43	3.42	0.54	0.55	3.51	3.52	0.10	0.10
Referee	3.29		0.38		3.61		0.07	
	3.32	3.31	0.34	0.36	3.55	3.58	0.07	0.07

[1] For report of Subcommittee B and action by the Association, see *This Journal*, **22**, 56 (1939).

examined under a magnification of 80 diameters these glycerol droplets may be plainly seen dispersed throughout the base. Sampling errors are very likely to occur with such a heterogeneous compound, particularly after exposure to extremes of temperature. To minimize this difficulty each batch of ointment was mixed well and stored in a 20° incubator.

The present tentative method for iodine and the proposed method for organically combined iodine were subjected to collaborative study. Two samples, one an ointment four years old labeled A, the other a freshly prepared ointment labeled B, were sent out with the request that they be mixed well before being analyzed. The results appear in the table.

Iodine.—No difficulty was reported by any of the collaborators with this procedure. Duplicate determinations of each worker show good agreement, and the small variation occurring among the several sets of results may be attributed to sampling errors or to time intervals occurring between analyses.

Organically Combined Iodine.—Although the results of a majority of the collaborators showed good agreement on the fresh sample, this was not the case with the older one. It was found very difficult to extract all the iodide from the base, in some instances more than thirty washings being required. It is apparent, therefore, that this procedure, while perhaps satisfactory for freshly prepared ointments, is unsuited for general work.

RECOMMENDATIONS[1]

It is recommended—

(1) That the present tentative method for the determination of iodine in iodine ointment be adopted as official, first action.

(2) That the study of methods for the determination of organically combined iodine in iodine ointment be discontinued.

REPORT ON THE SEPARATION OF ACETYLSALICYLIC ACID, ACETPHENETIDIN, AND CAFFEINE

By Donald C. Grove (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The problem of effecting a quantitative separation of acetylsalicylic acid, acetphenetidin, and caffeine in admixture has been studied by several investigators.

Methods of Analysis, A.O.A.C., 1935, 553, contains an official method for the determination of acetylsalicylic acid in mixtures containing acetphenetidin and caffeine. This method was developed by Harrison, *This Journal*, **8**, 499. It, however, is a method for determining only the acetylsalicylic acid, the acetphenetidin and caffeine both being discarded.

Hitchens[1] presented experimental data showing that acetylsalicylic acid could be separated satisfactorily from a number of medicinals, including acetphenetidin and caffeine by means of sodium bicarbonate. The acetylsalicylic acid could then be recovered by acidification of the bicarbonate solution and extraction with a suitable solvent.

Berman, *This Journal*, 19, 520 also effected a successful separation of the acetysalicylic acid from the acetphentidin and caffeine by means of sodium bicarbonate. For the separation of acetphenetidin and caffeine he tried several different methods, which did not prove very satisfactory in the hands of his collaborators.

The method as worked out in the present investigation employs the separation of acetylsalicylic acid from acetphenetidin and caffeine by means of sodium bicarbonate. The acetphenetidin and caffeine are then treated with a dilute solution of sulfuric acid, which removes all the caffeine plus a small quantity, usually about 75 mg., of acetphenetidin, leaving the main bulk of the acetphenetidin behind. The caffeine is then separated from the remaining acetphenetidin by means of the acid hydrolysis method of Emery, *This Journal*, 2, 63, which is a tentative method, *Methods of Analysis, A.O.A.C.*, 1935, 548.

Of the three ingredients to be determined, caffeine is most difficult to recover quantitatively. This is apparent for several reasons. First, the caffeine is present in the smallest proportion; second, it is the last ingredient determined and thus contains any errors of manipulative technic from the separation of the other two ingredients; third, if the hydrolysis of the acetphenetidin is not complete, any unconverted acetphenetidin will be weighed with it; and finally, extracted tablet lubricants may be weighed with the caffeine.

It is because of these conditions that a preliminary separation of the caffeine from as much of the phenacetin and tablet lubricants as possible, by means of weak sulfuric acid, was decided upon. The method also has the advantage of allowing a larger sample to be taken for assay and the small amount of phenacetin is hydrolyzed more completely.

The method that was sent out to collaborators was published in *This Journal*, 22, 91 (1939).

COLLABORATIVE WORK

Two samples were sent out for collaborative work. Sample No. 1 was a mixture prepared by the Associate Referee to contain acetylsalicyclic acid, 56 per cent; acetphenetidin, 40 per cent; and anhydrous caffeine, 4 per cent.

Sample No. 2 was a commercial compressed tablet stated by the manufacturer to contain, per tablet, acetylsalicylic acid, 0.1764 gram; acetphenetidin, 0.1176 gram; and caffeine, 0.0294 gram.

[1] *J. Am .Pharm. Assoc.*, 24, 1084 (1934).

TABLE 1.—*Collaborative results*
Sample No. 1.—Per cent

COLLABORATOR	ACETYLSALICYLIC ACID			ACETPHENETIDIN			CAFFEINE ANHYDROUS		
	FOUND	THEORETICAL	AV. RECOVERY	FOUND	THEORETICAL	AV. RECOVERY	FOUND	THEORETICAL	AV. RECOVERY
C. F. Bruening, Food & Drug Adm., Baltimore	54.58 54.65	56.00	*per cent* 97.5	41.23 41.01	40.00	*per cent* 102.8	4.14 4.10	4.00	103.0
H. H. Shull, McNeil Lab., Philadelphia	54.92 54.97	56.00	98.1	41.40 40.99	40.00	103.0	4.24 4.31	4.00	106.9
L. E. Warren, Food & Drug Adm., Washington	54.38 54.18	56.00	96.9	41.50 41.29	40.00	103.5	4.03 4.02	4.00	100.6
W. J. Watkins, The Upjohn Co., Kalamazoo	54.90 54.86	56.00	98.0	39.65 39.99	40.00	99.6	3.88 3.97	4.00	98.1
D. C. Grove	55.64 55.71	56.00	99.4	40.44 40.36	40.00	101.0	4.16 4.14	4.00	103.8
Average	54.88	56.00	98.0	40.79	40.00	102.0	4.10	4.00	102.5

Sample No. 2.—Gram/tablet

	ACETYLSALICYLIC ACID			ACETPHENETIDIN			CAFFEINE + H_2O		
	FOUND	DECLARED	AV. RECOVERY	FOUND	DECLARED	AV. RECOVERY	FOUND	DECLARED	AV. RECOVERY
C. F. Bruening	0.1785 0.1778	0.1764	*per cent* 101.0	0.1166 0.1174	0.1176	*per cent* 99.5	0.0301 0.0302	0.0294	*per cent* 102.6
H. H. Shull	0.1762 0.1784	0.1764	99.9	0.1166 0.1177	0.1176	99.6	0.0304 0.0303	0.0294	103.2
L. E. Warren	0.1764 0.1761	0.1764	99.9	0.1178 0.1180	0.1176	100.3	0.0303 0.0309	0.0294	104.1
W. J. Watkins	0.1744 0.1738	0.1764	98.7	0.1111 0.1092	0.1176	93.7	0.0292 0.0273	0.0294	96.1
D. C. Grove	0.1717 0.1726	0.1764	97.6	0.1190 0.1184	0.1176	100.9	0.0304 0.0304	0.0294	103.4
Average	0.1754	0.1764	99.4	0.1162	0.1176	98.8	0.0300	0.0294	101.9

The results and comments of the collaborators follow:

COMMENTS OF COLLABORATORS

C. F. Bruening.—The method gave no manipulative difficulties, but considerable time was involved in evaporating the phenetidin sulfate solutions until all the acetic acid was removed.

H. H. Shull.—It takes considerable time to run the samples by this method, but the results indicate that the method is the best we have tried for this separation. The method for aspirin is satisfactory as it stands. Titration of the residue indicates purity of 99.5 per cent aspirin. Some operators think that caffeine should be dried at 80° C. to constant weight, instead of at 100° C., to prevent loss.

W. J. Watkins.—The method we have been using for years on such mixtures is similar to yours and,—in our opinion, is reasonably satisfactory. It took considerable time to reach constant weight in drying the phenacetin after conversion from phenetidin, but aside from that your proposed method works well enough.

The agreement between collaborators is believed to be as satisfactory as could be expected in a mixture of this type, where all three ingredients are determined quantitatively on a single sample. Therefore it is recommended[1] that the proposed method for the determination of acetylsalicylic acid, acetphenetidin, and caffeine be adopted as tentative, and that the subject be closed.

REPORT ON GUMS IN DRUGS

By J. H. CANNON (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

During five years various methods for the identification of gums in drug products have been considered by the Associate Referee. Two years of collaborative study of tests depending upon the formation of characteristic precipitates from aqueous mixtures indicated that such a procedure is open to several objections, of which the most serious, perhaps, is that the concentration of gum present in an unknown is not readily ascertained, *This Journal*, 19, 528. This, together with the fact that the quality of certain precipitates seems to vary with the proportion of gum present, suggested that tests applied to the precipitated gum itself might be more dependable than precipitation reactions. Accordingly, a study was made by the Associate Referee of published tests of this type, principally those described in the literature of pharmacognosy and plant histology, and generally depending upon staining or color formation with the usual reagents used in microscopy.

Collaborative study on identifications of this type was made this year. Six unknowns were submitted, as follows: I (Irish moss), II (tragacanth), III (agar), IV (quince), V (karaya), and VI (galagum). Control samples of these gums and instructions to collaborators were sent with the unknowns.

The method was published in *This Journal*, 22, 92.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 57, 91 (1939) [1]

NOTE ON OUTLINE OF TESTS

Unlike the work on microchemical identification of alkaloids, the present scheme provides for confirmatory tests as well as elimination tests because, generally speaking, no single reaction is sufficient to identify any of the gums. In this connection the reactions of tragacanth may be cited as an illustration. With certain samples a rapid blue-black stain is produced with chlorzinc iodide. With other authentic samples the blue color appears more slowly. The microscopical characteristics of these two types are distinct. This possibly is due to differing methods of production of the crude gum, certain types being the knotty yellow or brown natural exudation, other types being the pure white or semi-transparent ribbons resulting from an incision in the bark of the shrub near the base. The latter is now said to be the favorite form, although the tragacanth of commerce is a conglomerate mixture of good, bad, and indifferent as obtained from the caravans.[1] There is also the possibility that various supposedly authentic samples of tragacanth may have been derived from different species of *Astragalus*, since, according to the literature, numerous species belonging to this genus yield a gummy matter having the properties of tragacanth.

Results of Collaborators

	I	II	III	IV	V	VI
F. J. McNall U. S. Food & Drug Adm. Chicago	Irish Moss	Traga-canth	Agar	Quince	Karaya	Gala-gum
E. C. Payne U. S. Food & Drug Adm. Chicago	Irish Moss	Traga-canth	Agar	Quince	Karaya	Gala-gum
J. Claggett Jones State Div. Chemistry Richmond	Irish Moss	Traga-canth	Acacia*	Quince	Karaya	Gala-gum
C. E. Shepard Conn. Agr. Exp. Sta. New Haven	Irish Moss	Traga-canth	Agar	Quince	Acacia*	Gala-gum
Waldo L. Scoville Mich. State Dept. Agr. Lansing	Irish Moss	Traga-canth	Agar	Quince	Acacia*	Gala-gum
J. Reilly U. S. Food & Drug Adm. New York	Irish Moss	Traga-canth	Agar	Quince	Karaya	Gala-gum

* These incorrect identifications were believed to be due to the use of too small a portion for testing. Accordingly, additional samples were submitted to these three collaborators, identified as VII, VIII, and IX. VII contained acacia, VIII contained karaya, and IX contained agar. All three collaborators identified these samples correctly.

[1] John Uri Lloyd, *Origin and History of Pharmacopial Vegetable Drugs*, 1921

COMMENTS BY COLLABORATORS

McNall.—Samples I, II, IV, and V were easily identified. With Sample III, I experienced some difficulty in getting a suitable precipitate free of mineral oil. The original precipitate came down fine and flocculent and after washing twice with ether it still contained enough oil to interfere with the test with tincture of iodine. The precipitate was again washed with ether, dissolved in water, and reprecipitated with alcohol. A mat was obtained which gave a good color reaction with iodine. The pink coloration with concentrated sulfuric acid and galagum is rather difficult to obtain since it is very fleeting.

Payne.—Little trouble was experienced when unknowns were compared directly with known samples. The characteristic structures in Irish moss were not always seen. The test somewhat resembles that for tragacanth. The pink color produced by hydrochloric acid on karaya may be easily missed but is definite. I would suggest that the reaction of every gum with all four group reagents be described in order to aid in sorting out.

Shepard.—All of the gums were stained pink with ruthenium red but probably karaya was stained deepest pink of all.

Jones.—The results were none too satisfactory. This was probably due to my lack of experience in this method of identification.

Scoville.—The appearance of the iodine test seemed to vary considerably, due apparently to the thickness and dryness of the mat. Sample V gave fine precipitate but did not appear granular. It seemed to dissolve in ruthenium red rather than be colored by it. The sulfuric acid group test does not appear to be as satisfactory as is desirable.

Reilly.—Reactions of the various gums with group I and group II reagents seem characteristic and should be useful for the identification of those gums. Tests depending on the use of ruthenium red reagent were found unsatisfactory, due perhaps to the condition of the reagent on hand.* The color change of galgagum with sulfuric acid is striking enough. Heating should be done cautiously. The behavior of acacia with this reagent seemed not so characteristic. The change to a greenish brown as described in the outline is not so readily perceptible, the color gradually darkening. When available, larger quantities of gum solution might be used to advantage.

SUMMARY AND RECOMMENDATIONS[1]

Collaborative results show that the method submitted is satisfactory for the six gums studied when these occur unmixed in drug preparations.

It must be noted that comparison with controls is essential, and that a certain degree of familiarity with the appearance of the different gum precipitates is an important part of the method.

It is the opinion of the Associate Referee that no further work on this subject is indicated at this time, accordingly it is recommended that the method be adopted as tentative and that the subject be closed.

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* This reagent decomposes slowly and it is believed that this accounts for the collaborator's statement on this point.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 57, 92 (1939).

REPORT ON THEOBROMINE IN THEOBROMINE CALCIUM TABLETS

By P. S. JORGENSEN (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

Study of this subject was continued this year in accordance with the recommendation of the referee. Samples of powdered theocalcin tablets, theocalcin powder, and theobromine alkaloid were sent to the collaborators with instructions to assay by the proposed acidimetric method and the present tentative iodometric method.

INSTRUCTIONS TO COLLABORATORS

Powder the sample in a mortar and dry at 110° C. Place 0.5 gram of the powdered tablets or 0.4 gram of the theocalcin powder, or 0.2 gram of theobromine alkaloid in a 300 cc. beaker and add 100 cc. of water. Warm moderately over a flame and add 15 cc. of approximately 0.1 N H_2SO_4. Heat to boiling to insure complete solution and to remove CO_2. Cool to room temperature under the tap. Add 1.5 cc. of phenol red indicator, render slightly alkaline with approximately 0.1 N NaOH, and titrate carefully to an acid reaction with 0.1 N H_2SO_4 (yellow color). To this solution add 25 cc. (an excess) of neutral 0.1 N $AgNO_3$ and titrate the liberated HNO_3 immediately with 0.05 N NaOH to a distinctly violet red color. Titrate cautiously drop by drop with constant stirring near the end point.

1 cc. of 0.05 N NaOH $= 0.009$ gram of $C_7H_8O_2N_4$.

The results obtained are given in the table.

COLLABORATOR	PER CENT THEOBROMINE IN THEOBROMINE ALKALOID		PER CENT THEOBROMINE IN THEOCALCIN POWDER		PER CENT THEOBROMINE IN THEOCALCIN TABLETS	
	ACIDIMETRIC METHOD	IODOMETRIC METHOD	ACIDIMETRIC METHOD	IODOMETRIC METHOD	ACIDIMETRIC METHOD	IODOMETRIC METHOD
M. L. Yakowitz	100.79	99.31	48.28	47.44	41.43	41.77
Food & Drug Adm.	100.28	98.66	48.69	47.48	41.53	41.06
San Francisco	100 96		48.30		41.49	
Harry Isacoff	99.87		48.69		41.29	
Mutual Pharmacal Co.	100.28		48.98		41.55	
Syracuse	99.94		48.92			
P. S. Jorgensen	99.94	103.18	49.16	50.67	41.81	44.55
	100.17	102.24	49.27	50.89	41.81	44.55

A study of the results shows that the iodometric method gives varying results, while the acidimetric method gives consistent results in the hands of different analysts. Furthermore, the acidimetric method is easily carried out in a few minutes while the iodometric method requires standing overnight. Considering then the better results obtained by the acidimetric method and the ease with which it may be performed, the Associ-

ate Referee feels justified in recommending[1] that this method replace the present tentative method.

REPORT ON CHLOROBUTANOL

By Frank C. Sinton (U. S. Food and Drug Administration,
U. S. Department of Agriculture, New York, N. Y.),
Associate Referee

Last year a collaborative study was made on the determination of chlorobutanol, and also chlorobutanol in aqueous solution. Although the results on the chlorobutanol crystals were reasonably in line with expectation, the findings on the solution showed marked discrepancies from the theoretical percentage of chlorobutanol. During the current year samples of chlorobutanol and chlorobutanol in solution were again submitted for collaborative study. The method for chlorobutanol was essentially the same as last year's, but in the method for chlorobutanol in solution some of the details of the distillation were changed.

The chlorobutanol used in the preparation of the collaborative samples was purchased from a reputable manufacturer and found to comply with the U.S.P. tests for purity. It was desiccated for four weeks and then found to have a melting point of 96° C.

The samples of chlorobutanol crystals were submitted to the collaborators in weighing bottles sealed with paraffin in order to avoid absorption of moisture. The sample of chlorobutanol in solution was of the same composition as that submitted to collaborators last year.

The method was published in *This Journal*, **22**, 95 (1939).

The results are shown in the table.

COMMENTS BY COLLABORATORS

Berman.—No manipulative difficulties were encountered. All solutions filtered before precipitation was made.

Hoshall.—The proposed method is well described, simple, and accurate, and no difficulty was experienced. We have been determining chlorobutanol by this method except that the chloride is determined volumetrically by the Volhard method instead of gravimetrically as silver chloride. Although the volumetric method may be a little more difficult from the manipulative standpoint, it is much more rapid than the gravimetric procedure.

Jorgensen.—The method is simple, and in my opinion constitutes a very good method for determining this substance.

Moraw.—Results on the solution do not check as well as I expected. Possible reasons that suggest themselves include the following: Incomplete saponification due to too much dilution of saponification solution, and incomplete washing down of condenser, adapter, etc.

I used a 22 inch condenser, but the experience with this suggests that a 14 inch condenser would be better because it would require less alcohol and water to

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, **22**, 57, 94 (1939).

thoroughly wash it down. Incidentally the use of small amounts of water as a follow-up wash after alcohol results in the water "rolling" through the condenser like oil, without wetting the condenser and without having its full washing effect. As one observes this he logically concludes he should use more water. I am skeptical as to whether the water wash is necessary at all.

	CHLOROBUTANOL	CHLOROBUTANOL IN SOLUTION (4.500 GRAMS/1000 CC)
	per cent	
S. M. Berman	99.46	4.35
Buffalo	99.53	4.32
Edward M. Hoshall	99.75	4.452
Baltimore	99.47	4.456
Paul S. Jorgensen	99.40	4.270
San Francisco	99.03	4.356
	98.65	4.331
H. O. Moraw	99.2	4.30
Chicago	99.6	4.39
		4.28
		4.29
F. A. Rotondaro	100.36	4.565
Philadelphia	99.61	4.573
C. A. Wood		4.39
New York		4 42
F. C. Sinton	99 8	4.48
	99.6	4.44
	99.6	4.45
Minimum recovery	98.65	4.27 (94.9%)
Maximum recovery	100.36	4.573 (101.6)
Average recovery	99.5	4.395 (97.7%)

For the determination of the chloride, the gravimetric and volumetric methods should be optional. If directions are given for either method they should agree in all respects with official or recognized procedures. For example, while it is doubtful if a difference of 5° in the drying temperature for the AgCl would make any difference in the results, since the U.S.P. directs drying it at 110° C. and Hillebrand and Lundell, 100° at first, then 130–150° C., it seems advisable to have your directions agree with one of these. Then the matter of acidity of the solution and precautions against too great excess of AgNO₃ and the washing solutions, temperature, standing, etc. should likewise be taken into consideration so as to conform more closely with recognized practice. Your directions for washing the AgCl differ from established methods.

The gravimetric method for AgCl is so widely published it should not be necessary to repeat it, but if reprinted it should be in agreement with the best authorities.

Rotondaro.—Since only an opalescence was obtained with the reagents used, no blank was deducted from the results.

No difficulty was encountered in the procedures outlined. Additional determinations were made in the case of the solution, 10 cc. of alcohol being added to the sample in addition to the 25 cc. of water. The resultant simultaneous distillation of chlorobutanol and alcohol kept the condenser and adapter clean. The washing of the condenser and adapter was then easily done with 5–10 cc. of alcohol and about 10 cc. of water. This modified procedure gave results of 99.68% in the chlorobutanol crystals and 0.4581 gram/100 cc. in the case of the solution.

CONCLUSION

Results obtained by the collaborators on the chlorobutanol crystals averaged 99.5 per cent, which constitutes a good recovery. In the case of the sample of chlorobutanol in solution the recoveries averaged close to 98 per cent of the theoretical, which appears to be quite reasonable for a determination of this nature.

The majority of the collaborators reported no difficulty with the method. In explanation of criticism by one of the collaborators regarding the method of determining the chloride it may be mentioned that for purposes of uniformity the method described in *Methods of Analysis, A.O.A.C.*, 1935, 579, for chloroform in mixtures was used for this purpose. No doubt any other official procedure, either volumetric or gravimetric, would be as accurate and acceptable since at this point the method involves simply the well known determination of chloride.

RECOMMENDATION[1]

It is recommended—

(1) That the method for the determination of chlorobutanol be adopted as tentative.

(2) That the method for the determination of chlorobutanol in solution be adopted as tentative.

(3) That the topic be discontinued.

REPORT ON PHENOLPHTHALEIN AND ACETYLSALICYLIC ACID

By George M. Johnson (U. S. Food and Drug Administration, Minneapolis, Minn.), *Associate Referee*

As a result of collaborative work done last year on this subject, the method was modified and a new mixture was prepared and sent out to collaborators for analysis. The mixture, made similarly to that used last year, contained 84.0 per cent of acetylsalicylic acid, 5.0 per cent of phenolphthalein, and approximately equal amounts of calcium carbonate, lactose, starch, and talc. The method used by the collaborators for the

determination of phenolphthalein and acetylsalicylic acid in tablets modified as later suggested by the Associate Referee was published in *This Journal*, **22**, 95.

The results obtained by the collaborators are given in the table.

Collaborative results

	ACETYLSALICYLIC ACID	RECOVERY	PHENOLPHTHALEIN (110°–120° C.)	RECOVERY AT 120°C.
	per cent	*per cent*	*per cent*	*per cent*
Henry R. Bond	75.07*		5.27*	
Food & Drug Adm.	80.27*		5.14*	
Chicago	79.65*		5.08*	
	76.27*		5.08*	
	74.93*		5.12*	
	83.33	99.2	4.83	96.6
	83.97	100.0	4.81	96.2
Jonas Carol	80.99	96.4	5.11	5.00	100.0
Food & Drug Adm.	81.03	96.5	5.20	5.12	102.4
Cincinnati	80.92	96.3	5.40	5.07	101.4
Irwin S. Shupe	82.7	98.5	5.52	5.12	102.4
Food & Drug Adm.	83.3	99.2	5.41	5.11	102.2
Kansas City				
McNeil Laboratories	84.1 H.H.S.	100.1	5.26	5.14	102.8
Philadelphia	83.2 H.H.S.	99.0	5.25	5.09	101.8
H. H. Schull	84.5 P.E.P.	100.6	5.41	5 16	103.2
Chief Chemist				
Llewellyn H. Welsh	82.83	98.6		5.03	100.6
Food & Drug Adm.	82.67	98.4		5.02	100 4
Baltimore	81.79*†			
	81.73*†			
C. A. Wood	82.9	98.7	5.22	5.16	103.2
Food & Drug Adm.	83.2	99.0	5.10	5.03	100.6
New York				
G. M. Johnson	83.64	99.6	5.14	5.06	101.2
	83.33	99.2	5.09	5.01	100.2
Average	82.91	98.7		5.05	101.0

* Not included in average.
† Samples accidentally allowed to evaporate to dryness on steam bath, although there was no acetous odor from decomposition.

The method is not specific on the details of the sodium bicarbonate extraction of the acetylsalicylic acid from the ether solution. For this reason two of the collaborators, Bond and Carol, obtained low results on the acetylsalicylic acid. Carol reports that he did not allow the bicar-

bonate solution to remain in contact with the ether solution except for a minimum time because of the possibility of hydrolysis of the acid. Bond conducted additional determinations, at the suggestion of the Associate Referee, allowing more time for the reaction between the acetylsalicylic acid and the bicarbonate to take place. These results on the acetylsalicylic acid are much closer to the actual content, although it is not known why low results were obtained on the phenolphthalein. If Carol's results on the acetylsalicylic acid are omitted, an average recovery of 99.2 per cent is shown, and with Bond's results on the phenolphthalein omitted, an average of 101.6 per cent is obtained, which results the Associate Referee considers a truer picture of the results possible by this method.

COMMENTS OF COLLABORATORS

Harry H. Schull.—The results check as closely as you would expect, considering the limitations of the gravimetric method. If the method is criticized because the end products weighed are not specific substances, it would be possible to determine the phenolphthalein as described on page 570, *A.O.A.C. Methods*, 1935, and the aspirin by one of the methods on page 551 of the same book.

Llewellyn H. Welsh.—The method entailed some manipulative difficulties because of the use of ether. In extraction of ether solution with 3 per cent alkali, there was a tendency toward seepage at the stopper and stopcock because of internal pressure.

A. C. Wood.—It is suggested that a titration might be used as a check on the purity of the aspirin residue. It also might be advisable to include a suitable warning in the method indicating that the bicarbonate extraction and isolation of the acid should be conducted as rapidly as possible in order to prevent hydrolysis.

DISCUSSION

The initial dry extraction of the sample is slow and tedious, but no solvent has been found that is an improvement on the ether. If sufficient time is allowed for neutralization of the acid by the sodium bicarbonate —each extraction being thoroughly shaken for about a minute—the solutions are kept cold, and the acetylsalicylic acid is extracted as soon as possible, practically quantitative results are obtainable.

Any acetylsalicylic acid not separated from the ether solution is eventually weighed as phenolphthalein, and it has been noticed that even with the most careful manipulation traces of salicylic acid are found in the phenolphthalein residue. It was for this reason that the Associate Referee suggested that the phenolphthalein residue be heated to constant weight at 120° C., rather than the customary 100° C., for at this temperature the traces of salicylic acid are volatilized.

It is recommended[1] that the method presented be adopted as tentative.

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, **22**, 57, (1939).

REPORT ON AMINOPYRINE AND PHENO-BARBITAL IN MIXTURES

By E. C. PAYNE (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The method reported on last year, *This Journal*, 21, 566–571, was tried by collaborators. The results, which are given in the table, were obtained with individually weighed samples of phenobarbital and aminopyrine. No excipients were added. For the preparation and purity of these substances see the same reference. The results follow:

ANALYST	EXCIPIENT	SUBSTANCE	TAKEN	RECOVERED	RECOVERY
			gram	*gram*	*per cent*
H. O. Moraw	None	Aminopyrine	0.3037	0.3023	99.5
H. O. Moraw	None	Phenobarbital	0.1031	0.1038	100.7
H. O. Moraw	None	Aminopyrine	0.4087	0.4071	99.7
H. O. Moraw	None	Phenobarbital	0.1545	0.1540	99.6
R. D. Stanley	None	Aminopyrine	0.3770	0.3743	99.3
R. D. Stanley	None	Phenobarbital	0.1046	0.1051	100.5

In addition, the following simple methods of separation were tried:

(1) *"Dry" extraction with CCl_4.*—Phenobarbital is slightly soluble in this solvent and it is therefore unsuitable.

(2) *"Dry" extraction with petroleum ether.*—While it appears that this solvent will eventually remove the aminopyrine, so many extractions are necessary that the process becomes unduly tedious. For example, twenty 30 cc. portions of solvent did not completely remove 0.4 gram of aminopyrine from 0.2 gram of phenobarbital.

(3) *Extraction of phenobarbital from acid solution ($1+9$ H_2SO_4) with chloroform or ether, and later removal of aminopyrine after making alkaline with ammonia.*—Samples of 0.1–0.2 gram of phenobarbital and 0.2–0.5 gram of aminopyrine were shaken with 25 cc. of the acid and the solution was extracted with the solvent until a test portion yielded no weighable residue. The solvent was washed with 5–10 cc. of water, which was added to the aqueous layer, filtered into a tared beaker and evaporated, and the residue was dried and weighed. The aqueous solution was made alkaline with ammonia and the aminopyrine extracted with chloroform, etc. The results are shown in the following table:

NO. OF SAMPLES	SOLVENT FOR PHENOBARBITAL	RECOVERY OF PHENOBARBITAL (AV.)	SOLVENT FOR AMINOPYRINE	RECOVERY OF AMINOPYRINE (AV.)
		per cent		*per cent*
8	Ether	102.4	Chloroform	96.6
3	Chloroform	102.6	Chloroform	98.5

The results indicate that separation is incomplete. Strangely enough, further extraction of the original acid solution with solvent yields no more extract. This might be explained by a slight interaction between aminopyrine and phenobarbital, a phenomenon known to occur at a somewhat higher temperature. Not enough results are yet available for definite conclusions to be drawn.

A somewhat different method was tried by L. E. Warren. He used the above method for aminopyrine, but extracted the phenobarbital in a continuous extractor, using chloroform as solvent. When he used individually weighed samples containing no excipients, his method gave 100.1–100.3 per cent recovery for aminopyrine and 100.4 per cent for phenobarbital. With similar samples containing lactose and starch he obtained 100.2 per cent recovery for aminopyrine and 101.5–102.3 per cent for phenobarbital.

In view of the small number of collaborative results and of the absence of excipients in the samples, the Associate Referee recommends[1] that the problem be reassigned to an associate referee for a study of the method when applied to tablets and other medicinal mixtures.

REPORT ON ELIXIR OF TERPIN HYDRATE AND CODEINE

By JONAS CAROL (U. S. Food and Drug Administration,
Cincinnati, Ohio), *Associate Referee*

Last year a collaborative study of a method of analysis of elixir of terpin hydrate and codeine was made. Despite the small amount of codeine present in the elixir, very favorable recoveries were made by most of the collaborators. The results on terpin hydrate, however, were less satisfactory, being too high in all cases. Most of the collaborators considered that these high results were caused by condensation of moisture during the evaporation of the extract solution containing the terpin hydrate. This moisture apparently was not lost by subsequent drying of the residue without heat. The use of only a moderate amount of heat (50° C.) caused a constant loss of weight of the terpin hydrate residue. This was probably the result of loss of water of hydration in addition to water gained by condensation.

This year an attempt was made to find a satisfactory method of obtaining a dry terpin hydrate residue. In a preliminary study, aliquots of a solution of terpin hydrate in alcohol-chloroform solvent (7 per cent alcohol) were evaporated both by a current of air so as to condense moisture, and spontaneously. The residues obtained were then left exposed to air and weighed daily for one week. Results are shown in Table 1.

TABLE 1.—*Results on finding dry residue*

(Aliquots contained 0.1704 gram of terpin hydrate in 100 cc. 7% alcohol-chloroform mixture)

DAYS	SAMPLES EVAPORATED IN CURRENT OF AIR		SAMPLES ALLOWED TO EVAPORATE SPONTANEOUSLY	
0*	0.1731	0.1733	0.1715†	0.1711†
1	0.1731	0.1733	0.1715	0.1707
2	0.1729	0.1726	0.1702	0.1704
3	0.1724	0.1724	0.1709	0.1704
4	0.1728	0.1729	0.1705	0.1700
5	0.1726	0.1729	0.1703	0.1701
6	0.1725	0.1726	0.1703	0.1701
7	0.1725	0.1727	0.1702	0.1700

* Weighings began when residue was apparently dry.
† 35 hours from beginning of evaporation.
The weather during this week ranged from rainy and wet to hot and dry.

The results, Table 1, indicate (1) that contrary to the accepted view terpin hydrate loses water extremely slowly when exposed to air, and (2) that moisture gained by the residue during condensation is not lost again by exposure to air.

With the hope that spontaneous evaporation of the terpin hydrate extract was the solution to the problem, samples of elixir of terpin hydrate and codeine N.F. VI (containing 17 grams of terpin hydrate and 2 grams of codeine alkaloid per 1000 cc.) were sent to the collaborators to be analyzed by the following method:

METHOD

Terpin Hydrate.—Measure a 10 cc. sample from a small buret (allow to drain 5 minutes) into 10 cc. of water in a separator. Add 2 cc. of 10% H_2SO_4. Immediately extract (on standing crystals form and cause some inconvenience) with two 10 cc. portions of petroleum benzin and wash the combined petroleum benzin extracts with three 2 cc. portions of water to which 3–4 drops of dilute H_2SO_4 have been added. Petroleum benzin contains aromatics and may be discarded. Return acid washings to original separator and extract completely with alcohol-$CHCl_3$ solution (7% alcohol) to remove the terpin hydrate. (Seven extractions of 20 cc. portions should be sufficient.) Make an additional extraction and evaporate to dryness to test for complete extraction of terpin hydrate. Wash the combined alcohol-$CHCl_3$ extracts in a second separator containing 10 cc. of 2% H_2SO_4. (This wash is very important as glycerol carried over from original sample by $CHCl_3$-alcohol solution must be removed to prevent its being weighed with the terpin hydrate. The washing also prevents loss of codeine.)

Filter the alcohol-$CHCl_3$ extract through a pledget of cotton, previously wet with $CHCl_3$, into a tared crystallizing dish. Wash the dilute acid remaining in the separator with 10 cc. of the alcohol-$CHCl_3$ solvent, filter through cotton and add to the bulk of the solvent. Allow the combined solvent to evaporate spontaneously. (Experiments indicate that constant weight is reached in about 35 hours.) Report as terpin hydrate gram/100 cc.

Codeine.—Transfer the acid wash material to the original separator and make alkaline with ammonia. Determine codeine by the A.O.A.C. method 565, 69, be-

ginning line 5 ". . . extract 5 times with $CHCl_3$ . . . ." Report as gram/100 cc. of codeine. 1 cc. of 0.02 N H_2SO_4 = 0.00634 gram of $C_{18}H_{21}O_3N \cdot H_2O$.

Table 2 contains the results obtained by the collaborators and the Associate Referee.

TABLE 2.—*Collaborative results on terpin hydrate and codeine*

COLLABORATOR	TERPIN HYDRATE		CODEINE	
	G./100 cc.	% RECOVERY	G./100 cc.	% RECOVERY
C. B. Stone	1.701	100.1	0.199	99.5
Cincinnati	1.715	100.9	0.199	99.5
H. W. Conroy	1.750	102.9	0.203	101.5
Minneapolis	1.745	102.6	0.203	101.5
H. G. Underwood	1.719	101.1	0.198	99.0
Chicago	1.708	100.5	0.197	98.5
L. H. Welsh	1.742	102.5	0.200	100.0
Baltimore	1.735	102.1	0.198	99.0
	1.738*	102.2	0.198	92.0
	1.754*	103.2		
R. Hyatt	1.763	103.7	0.202	101.0
Cincinnati	1.767	103.9	0.200	100.0
Jonas Carol	1.697	99.8	0.201	100.5
Cincinnati	1.705	100.3	0.202	101.0
	1.709	100.5		
M. L. Yakowitz	1.790	105.3	0.180	90.0
San Francisco	1.768	104.0	0.187	93.5
I. S. Shupe	1.71	100.6	0.190	95.0
Kansas City	1.73	101.8	0.190	95.0
L. E. Warren	1.728	101.7		
Washington				

* Obtained by evaporating almost to dryness on the hot stone of a steam bath in a current of air, then removing dishes from steam bath and allowing them to stand in current of air for one hour.

The results again show that most of the analysts obtained very good recovery of codeine.

The results for terpin hydrate were appreciably better than the results reported last year. While Hyatt was making his determination the weather was very wet and rainy at Cincinnati, which might have caused his rather high result. Possibly moist weather at San Francisco also caused the high figure obtained by Yakowitz.

It is recommended[1] that the method be adopted as a tentative method.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 57 (1939).

REPORT ON EMULSIONS

By W. F. KUNKE (U. S. Food and Drug Administration,
Chicago, Ill.), *Associate Referee*

Experimental work was continued as indicated in the report of last year and as recommended by Subcommittee B, *This Journal*, 21, 67. The limiting conditions of certain analytical details were determined and the proposed method was studied collaboratively. It is now recommended as a tentative method.

Last year it was shown that Soxhlet extraction with chloroform and with powdered pumice as a "spreader" gave incomplete extraction, 97.4 per cent recovery, even after a 9-hour period coupled with siphoning every 5 minutes, following standing overnight with chloroform in the extraction apparatus.

This year it was found that continued Soxhlet chloroform extraction (8½ hours) of the ingredients of the emulsion other than cod liver oil, namely, 0.5 gram of acacia, 1.5 cc. of water, and 0.4 cc. of sirup mixed with 20 grams of powdered pumice, yielded 0.0125 gram of residue after evaporation of the chloroform. This would introduce an error of +0.6 per cent on 2 grams of cod liver oil in a sample of an emulsion if the combined residue was not subsequently purified and assuming complete recovery of cod liver oil. Furthermore, when cod liver oil, 2.2773 grams, was dissolved in chloroform and the solution was refluxed in an acetylization flask on a steam bath for 10 hours, the cod liver oil, after evaporation of the chloroform, showed an increase in weight of 0.44 per cent. This was confirmed by another result of 0.45 per cent. Because of these unexpected results: (1) the material extracted when no oil was present, (2) the increase in weight of the oil due to a long period of refluxing, and (3) the low recovery, the Soxhlet extraction was abandoned.

Other methods of extraction of the cod liver oil from an emulsion were tried. (1) Extraction with chloroform of the emulsion previously mixed with a comparatively large volume of water was hopeless because of the very troublesome emulsion (strongly acidifying the mixture with hydrochloric acid did not improve matters). (2) The emulsion of cod liver oil was treated by mixing with 10 cc. of absolute alcohol, by stirring and evaporating the alcohol on a steam bath in the hope of "breaking" the emulsion in preparation for the extraction of the oil with chloroform (recovery was 90.6 per cent and 96.4 per cent). (3) The cod liver oil was extracted from the emulsion previously mixed in a beaker with a "spreader" to facilitate the extraction with chloroform (the most promising).

Various "spreaders" were tried, namely, powdered pumice, small pieces of filter paper, filter-cel, sodium chloride, magnesium oxide, crystalline calcium carbonate, and finely powdered calcium carbonate. Sodium chloride, magnesium oxide, and crystalline calcium carbonate were

unsatisfactory. Typical results for recovery of the cod liver oil from an emulsion by extraction with chloroform and the use of the various "spreaders," were: powdered pumice 99.8 per cent, filter paper 99.0–100.1 per cent, and filter-cel 100.1–100.8 per cent. Powdered pumice retains a comparatively large volume of chloroform and therefore a large volume of the solvent is necessary for extraction and washing; filter paper tends to form large masses, which may in some cases cause troublesome and incomplete extraction; and filter-cel was found to contain some chloroform-soluble material, which may account for the high results obtained. Filter-cel purified by removing the very small proportion of chloroform-soluble material is preferred next to finely powdered calcium carbonate, which is the best "spreader" and accordingly is specified in the proposed method. A recovery of 99.8 per cent is typical. In each case sufficient of the "spreader" was used with the emulsion sample to give practically a dry mixture in preparation for extraction of the oil with chloroform.

ABSORPTION OF OXYGEN BY COD LIVER OIL

It was shown experimentally that when 2.0000 grams of cod liver oil is dissolved in 100 cc. of chloroform and the chloroform is evaporated on a steam bath with the aid of a current of air, no increase in weight of the cod liver oil occurs. Since the proposed method directs weighing of the oil in a tared beaker, the conditions of drying that would cause a negligible or no increase in the weight of the oil were determined. The results of the various experiments are given in Table 1.

TABLE 1.—*Increase in weight of cod liver oil*

Conditions and period of heating

COD LIVER OIL*	NO HEATING, (ROOM TEMP.)	ELECTRIC OVEN		STEAM BATH, WITH CURRENT OF AIR	GAIN IN WEIGHT
		80° C.	100° C.		
grams	*days*	*hours*	*minutes*	*minutes*	*per cent*
1.2870	—	—	—	20	0.0
	—	—	—	50	0.07
	—	—	—	95	0.40
1.8715	—	—	20	—	0.0
	—	—	55	—	0.06
	—	—	95	—	0.08
2.0000	—	2	—	—	0.05
	—	48	—	—	3.5†
1.8050	3	—	—	—	0.8
	5	—	—	—	3.2†

* In an open 100 cc. beaker.
† Oil dark yellow and viscous.

It will be seen that the drying periods of 10 minutes on the steam bath with a current of air followed by 5 minutes at 100° C., as specified in the proposed method, is well within the safe limits to avoid an increase in weight or absorption of oxygen.

Besides influencing the weight of the oil, it is well known that exposure of fatty oils to atmospheric oxygen changes the analytical constants—the iodine value is decreased, the index of refraction increased, and the acidity may also increase. This is of extreme importance in case the analytical constants of the extracted oil are to be determined for the purpose of detecting inferior quality or adulteration of the cod liver oil used in preparing the emulsion.

PREPARATION OF THE EMULSION OF COD LIVER OIL

A sample of the emulsion in an open beaker loses weight upon standing at room temperature. This has a direct bearing on the cod liver oil content of an emulsion, because during its preparation the evaporation of some of the water may be sufficient to cause the finished emulsion to have a higher oil content than would be expected from the weights of the ingredients used. After various trials, the best procedure for preparing a small sample of a U.S.P. emulsion (about 4 grams), was found to be the English method, in which the acacia is dissolved in the water, the cod liver oil is added in small portions and thoroughly mixed to a thick, homogeneous emulsion, and the sirup is added and likewise mixed. In order to be able to determine definitely whether a loss in weight occurs due to the evaporation of some of the water during the preparation of an emulsion, the sample was prepared in a tared 100 cc. beaker. A tared stirring rod was used, the ingredients were weighed on a quantitative balance, a weighing buret was used for the oil, and the amounts of the ingredients were: acacia 0.5000 gram, water 1.5280 gram, cod liver oil 2.3915 grams, and sirup 0.5210 gram (alcohol and methyl salicylate were not used). The loss in weight during the preparation of the emulsion, or the difference between the sum of the weights of the ingredients and the weight of the finished emulsion, was found to be 6.7 per cent (this loss would vary in different lots). Doubtless a loss would also occur during the preparation of a large batch of emulsion. Such loss can not be readily and accurately determined, consequently the oil content of a large batch of an emulsion can not be accurately known from the weights of the ingredients.

Obviously, for the purpose of devising a method, the oil content of the emulsion should be accurately known; therefore, the entire amount of a small batch of emulsion (about 4 grams, prepared as given above) was used for each determination made by the collaborators and associate referee. The exact weight of the cod liver oil in each sample was known, and the oil was weighed by means of a weighing buret. In this experimental work, the exact weight of the oil alone is significant. Only the

approximate weight of each of the other ingredients was known and the total weight of the sample of emulsion was not taken into consideration.

Table 2 gives the results of collaborative study of the proposed method. The Associate Referee prepared each sample, and the entire sample was used for each determination. The collaborative samples, in form of an emulsion, contained besides the quantity of cod liver oil given, varying quantities of acacia, water, and sirup.

TABLE 2.—*Results of collaborative study of the proposed method*

COLLABORATOR	COD LIVER OIL		
	PRESENT*	FOUND	RECOVERY
	grams	*grams*	*per cent*
E. H. Berry	2.0165	2.0070	99.5
	2.0154	2.0047	99.5
	2.0530	2.0436	99.5
R. Jinkins	2.0720	2.0691	99.9
	2.0257	2.0177	99.6
H. O. Moraw	2.1697	2.1729	100.1
	2.0110	2.0146	100.2
R. S. Stanley	2.0250	2.0289	100.2
	2.0140	2.0124	99.9

* The U.S.P. cod liver oil was weighed by means of a weighing buret and incorporated in an emulsion.

EMULSION CONTAINING GLYCEROL AND ALCOHOL

Some emulsions on the market that contain cod liver oil also contain glycerol and alcohol. Experimental results obtained by the Associate Referee for carefully prepared samples of such an emulsion show that the proposed method will give good results. The four varied from 100.0 to 100.4 per cent recovery of the oil incorporated, about one-half as much glycerol as cod liver oil was used, and the alcohol content was about 7 per cent.

The method was published in *This Journal,* **22,** 96 (1939).

It is recommended[1] that the proposed method be adopted as a tentative method, and that the subject, in regard to the emulsion of cod liver oil, be closed.

[1] For report of Subcommittee B and action by the Association, see *This Journal,* **22,** 57, 96 (1939).

REPORT ON OINTMENT OF MERCURIC NITRATE
(CITRINE OINTMENT)

By H. O. MORAW (U. S. Food and Drug Administration,
Chicago, Ill.), *Associate Referee*

At the 1937 meeting the Associate Referee reported results of his investigation of possible methods and collaborative results on a proposed method. The method was based on separation of the mercury from the ointment base by 1+1 nitric acid digestion followed by acid permanganate digestion to insure that the mercury is in the bivalent state, eliminate oxides of nitrogen, and reduce organic matter; and titration of the mercury with standard thiocyanate. The results of the majority of the collaborators were within 3 per cent or less of the theoretical. The Committee recommended further study to improve the accuracy of the method.

Accordingly, the Associate Referee investigated the following points during 1938, and resubmitted the method to collaborators with a known sample. There were minor changes in the directions, but the principles of the method were the same as in 1937.

(1) Precautions necessary to insure the maintenance of an excess of permanganate during the acid permanganate digestion.

(2) Possible losses of mercury by volatilization during the digestions.

(3) Whether boiling during the 1+1 HNO_3 digestion is required or heating on steam bath is sufficient.

(4) Necessity of providing additional HNO_3 for the permanganate digestion.

The results of these investigations are shown in Table 1.

PURITY OF THE MERCURY

Two solutions designated A and B were prepared; redistilled mercury dissolved in nitric acid was used. Solution A consisted of 14.1050 grams of the mercury dissolved in 30 cc. of nitric acid and diluted to 1 liter. This was used chiefly for determining the purity of the mercury and checking on possible losses by volatilization. An average of four determinations showed the mercury to be 99.87 per cent pure. Solution B consisted of 31.1996 grams of mercury weighed in a tared glass-stoppered flask to which was added 44.57 grams of nitric acid (proportion required by the N.F. formula for preparing the mercuric nitrate used in making citrine ointment). After the reaction was completed in the open flask, it was stoppered and allowed to cool. It was found to weigh 70.9131 grams, which corresponded to 44.0 per cent crude mercury content. The mercury found in this solution by assay was 43.84–43.94 per cent, or an average of 43.88 per cent. Two uses were made of this solution, namely, mixing accurately weighed portions with appropriate quantities of nitrated lard to prepare assayable amounts of citrine ointment for use in checking the dependability of the proposed method, and for preparing the collaborative samples.

TABLE 1.—*Investigational results on known citrine ointment*

VARIATIONS FROM METHOD	NITRATED LARD*	MERCURIC NITRATE* SOLN. B	Hg PRESENT	Hg FOUND	RECOVERY	COMMENT
	grams	*grams*	*grams*	*grams*	*per cent*	
1. As proposed in 1937	5.5	0.9758	0.4282	0.4278	99.9	Satisfactory
2. As proposed in 1937 except no more HNO₃ added for KMnO₄ digestion		0.9758	0.4282	0.4195	98.0	Solution was the remaining half from first digestion in Determination 1
3. As proposed in 1937 except no more HNO₃ added for KMnO₄ digestion	5	0.7900	0.3467	0.3427	98.9	Half of HNO₃ digestion used
4. As proposed in 1937		0.7900	0.3467	0.3465	99.9	Remaining half of HNO₃ digestion from Determination 3
5. As proposed in 1937	3	0.7826	0.3435	0.3423	99.7	Satisfactory
6. 1st or HNO₃ digestion on steam bath	4	0.8431	0.3700	0.3554	96.1	Low result apparently due to failure to boil
7. 1+1 HNO₃ digestion in 200 cc. Erlenmeyer, gentle boiling	5	0.9194	0.4034	0.4016	99.5	Satisfactory
8. As proposed for 1937 HNO₃ digestion in Kjeldahl	4.2	0.7220	0.3168	0.3143	99.2	Satisfactory
9. 1+1 HNO₃ digestion in 200 cc. Erlenmeyer	4.0	0.9149	0.3934	0.3932	100.0	Satisfactory
10. 1+1 HNO₃ digestion in 200 cc. Erlenmeyer	4.0	1.5882	0.6829	0.6813	99.8	Satisfactory
11. 1+1 HNO₃ digestion in 200 cc. Erlenmeyer. 15 cc. H₂SO₄ added to half of soln and digested to fuming	5.0	0.9194	0.4034	0.3921	97.1	This experiment was on half of the 1+1 HNO₃ digestion from Determination 7.

* Same as required for the N.F. product; they were mixed in the digestion flask by gentle warming and then used for assays.

DISCUSSION OF ABOVE EXPERIMENTAL RESULTS

Heating the 1+1 nitric acid digestion on the steam bath apparently does not effect complete extraction of the mercury. Gentle boiling seems to be necessary. The additional nitric acid specified in the method for the potassium permanganate digestion is necessary to effect complete recovery. The use of the Kjeldahl flask for the 1+1 nitric acid digestion does not seem to be necessary, since the recoveries were equally as good with the 200 cc. Erlenmeyers. From the recoveries obtained it may be assumed that there is no detectable loss by volatilization during either of the digestions as directed in this method.

TABLE 2.—*Volatility of mercury from* $Hg(NO_3)_2$ *solution*

(No organic matter present)

DET. NO.	SAMPLE AND TREATMENT	HG PRESENT	HG FOUND	RECOVERY
		grams	*grams*	*per cent*
1	25 cc. Soln A in 500 cc. Kjeldahl +40 cc. 1+1 HNO₃, digested 2 hrs. Final volume, 40 cc.	0.3521	0.3517	99.9
2	25 cc. Soln A in 500 cc. Kjeldahl +40 cc. 1+1 HNO₃, digested 2 hrs. Final volume, 40 cc.	0.3521	0.3502	99.5
3	25 cc. Soln A in 250 cc. lipped Erlenmeyer +40 cc. 1+1 HNO₃ digested 1 hr. Final volume 40 cc.	0.3521	0.3522	100.0
4	25 cc. Soln A in 250 cc. lipped Erlenmeyer +40 cc. 1+1 HNO₃ digested 1½ hrs. Final volume 15 cc.	0.3521	0.3522	100.0
5	25 cc. Soln A+40 cc. 1+1 HNO₃ +15 cc. H₂SO₄ digested to fuming	0.3521	0.3543	100.7*

* End point not normal.

Experiment 11 (Table 1) shows one of the conditions (not applicable in this method) under which there is a slight loss of mercury by volatilization. In this experiment half of the nitric acid digestion from Experiment 7 was digested to fuming after sulfuric acid had been added, and the recovery was only 97.1 per cent compared with 99.5 per cent in Experiment 7, which was the other half of the solution treated according to the method. This table presents the results of 7 determinations averaging 99.7 per cent recovery after the samples of authentic citrine ointment had been digested about 1½ hours with 1+1 nitric acid and filtered, and the filtrate digested 30–45 minutes with HNO₃-H₂SO₄-KMnO₄. The recovery was about the same whether the first digestion was conducted in 500

cc. Kjeldahl or 200 cc. Erlenmeyer flasks with short-stemmed funnels in the necks. In all cases the second digestions were made in open 500 cc. Erlenmeyer flasks.

Solutions of mercuric nitrate in nitric acid and in nitric-sulfuric acids were digested (in the absence of organic matter) in 500 cc. Kjeldahl and 200 cc. Erlenmeyer flasks from volumes starting with 65 cc. of 1+2 nitric acid to final volumes of 40 cc. and 15 cc. Complete recovery of the mercury was obtained as shown in Table 2. Experiment 6 in this table shows the result of digesting to fuming after adding sulfuric acid. There was no organic matter present in this case, and although not conclusive the result, when considered with Experiment 11 in Table 1, suggests that the loss in the latter case was connected with the organic matter present.

TABLE 3.—*Collaborative results*

COLLABORATOR	OMITTING TEST FOR COMPLETE EXTRACTION OF HG FROM FAT		ADDING TEST FOR COMPLETE EXTRACTION OF HG FROM FAT	
	HG FOUND	RECOVERY ON 7.28% PRESENT	HG FOUND	RECOVERY ON 7.28% PRESENT
	per cent	*per cent*	*per cent*	*per cent*
E. H. Grant, U. S. Food & Drug Adm., Boston			7.39	101.5
			7.34	100.8
C. B. Stone, U. S. Food & Drug Adm., Cincinnati	7.33	100.7	7.30	100.3
	7.33	100.7	7.30	100.3
Rupert Hyatt, U. S. Food & Drug Adm., Cincinnati	7.44	102.3	7.38	101.4
	7.42	101.9	7.39	101.5
K. L. Milstead, U. S. Food & Drug Adm., St. Louis	7.37	101.2	7.29	100.1
	7.35	101.0	7.31	100.4
Wm. F. Reindollar, Maryland Bur. Chemistry, Baltimore	7.20	98.9	7.14	98.1
	7.03*	96.6*	6.88*	94.5*
	7.26	99.7	7.24	99.5
E. H. Berry, U. S. Food & Drug Adm., Chicago	7.22	99.2	7.22	99.2
	7.26	99.7	7.22	99.2
H. O. Moraw, U. S. Food & Drug Adm., Chicago	7.29	100.1	7.26	99.7
	7.30	100.3	7.26	99.7
L. E. Warren, U. S. Food & Drug Adm., Washington	7.39	101.5	7.38	101.4
	7.40	101.6	7.39	101.5
			7.43	102.0
			7.42	101.9
			7.39	101.5
Average of all results	7.31	100.4	7.30	100.2

* Average omitting lowest.   7.32   100.6   7.32   100.5

The method submitted to collaborators was published in *This Journal,* **22,** 96 (1939).

COMMENTS BY COLLABORATORS

E. H. Grant.—(Submitted too late for publication 1937): The directions should call for preparing the 3% peroxide from the 30% article, The acetanilid present in the commercial product may form a yellow color, which interferes with the end point.

Rupert Hyatt.—The test for complete extraction was practically negligible, and does not appear to be necessary. Shorter heating periods, i.e., boiling 5 minutes with the 1+1 HNO_3 and 10 or 15 minutes for the $KMnO_4$ digestion should suffice. Stone and I mixed our samples before beginning the analysis. Since we obtained checks on ourselves, the difference is apparently due to sample or personal equations.

K. L. Milstead.—By trial I found that the test for complete extraction required about the same volume of 0.1 *N* NH_4CNS whether diluted to 200 cc. and only 100 cc. used or whether the entire test is carried through the $KMnO_4$ digestion, i.e., about 1 minim or 0.05 cc. at most. A blank on the reagents required only a fraction of a drop. Even when varying amounts of sample were used, the test for complete extraction required the same volume of thiocyanate. I believe the test for complete extraction is unnecessary and the additional titration is not due to Hg.

E. H. Berry.—On testing for complete extraction 0.1 cc. was required on one determination and none on the other.

Wm. F. Reindollar.—Although the amount of permanganate consumed seems inordinately large, the method presents no difficulties when followed closely.

SUMMARY

Results are submitted showing an average recovery of 99.7 per cent of the mercury in seven experiments by applying the proposed method to authentic individual assayable quantities of citrine ointment, each prepared separately from accurately weighed amounts of mercuric nitrate of known mercury content and mixed with nitrated lard; each of these was prepared as required by the N.F. for the official citrine ointment. Attention is directed to the fact that in Experiments 1 and 2, 3 and 4, 7 and 11, one-half of the nitric acid extract was used for completing the determination as directed by the method and the other half used for a modified method. In this way the effect of the variation could be attributed to no other cause than the variation itself.

These results should remove doubt concerning the supposed pitfalls logically applicable to mercury digestions, such as incomplete extraction of the mercury from the ointment base, loss of mercury by volatilization, and titration of all the mercury.

The finished weight of the batch of ointment prepared for collaborative work was 280.41 grams and it contained 20.411 grams of mercury or 7.28 per cent. The majority of the collaborative results did not vary more than ±0.1 per cent from this amount, and the average of 14 results, 7.31 per cent, is in good agreement. Whether the variations indicate non-uniformity of the collaborative sample or reflect the personal factor can not be decided. If it is conceded that analytical accuracy may not be

attained in preparing large amounts of a product such as the 280 grams for collaborative study and that the true mercury content was not 7.28 per cent, it nevertheless appears that the uniformity of the collaborative results is reasonably good and that they indicate approximately the correct amount.

Two of the collaborators, Milstead and Hyatt, do not believe the test for complete extraction is necessary and Milstead points out that the same volume of 0.1N ammonium sulfocyanate is required for the test irrespective of size of sample, and about the same for a blank. It was the belief of the Associate Referee based on 1937 tests that this test was unnecessary. However, since the personal equation may enter into application of the method and it is the analyst's responsibility to insure complete recovery, this test was incorporated in the 1938 directions. Further reflection on this point in connection with comments by the collaborators indicates that the titration from the test should not be added to the main titration unless it is greater than a blank titration.

It is recommended[1] that the method be adopted as a tentative method with a view to making it official and that the subject be closed.

No report on rhubarb and rhaponticum was given by the associate referee. _____

No report on theophylline sodium salicylate was given by the associate referee. _____

REPORT ON SULFANILAMIDE

By Edward M. Hoshall (U. S. Food and Drug Administration, Baltimore, Md.), *Associate Referee*

The widespread use of sulfanilamide (para-aminobenzenesulfonamide) in the treatment of various bacterial infections in humans and the dangers associated with its indiscriminate use make it desirable to have an accurate method for its determination in tablets and to a lesser extent in the other forms in which it may be dispensed. At the present time practically all the product is available only in tablet form, either with suitable tablet excipients or with equal quantities of sodium bicarbonate.

Methods for the analysis of sulfanilamide may be based on any one of the following general types:

1. Determination of carbon, hydrogen, and nitrogen by combustion analysis.
2. Determination of nitrogen by the Kjedahl method.[2]
3. Determination of sulfur by Messenger's[3] or other methods.

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 58, 96 (1939).
[2] *Methods of Analysis*, A.O.A.C., 1935, 23.
[3] *Quart. J. Pharm. Pharmacol.*, 9, 560 (1936).

4. Determination of the nuclear-attached amino group by diazotization and observing the end point with starch-iodide paper,[1] or diazotization and subsequent coupling with organic reagents to form azo dyes, which are then determined colorimetrically.[*,2,3]
5. Determination by mercuration.[4]
6. Determination by bromination of the nucleus.[5,6]
7. Determination of the sulfonamido group.[5]

<div align="center">PART I</div>

<div align="center">EXPERIMENTAL</div>

Pure sulfanilamide, M.P. 166.0° C. was obtained. It was twice recrystallized from boiling alcohol and dried under vacumn and finally over sulfuric acid. The melting point remained constant. It was then examined for contaminants by the tests proposed by the A. M. A. Chemical Laboratory[1] and found to conform to their standards of identity and purity in all respects. Nitrogen determined by the A.O.A.C. Kjeldahl method was equivalent to a purity of 99.8 and 99.9 per cent of sulfanilamide. This material was used as a standard in all the experimental work and in the preparation of the collaborative samples.

The first three general methods were adjudged to be lacking in specificity and accordingly no work was carried out on these methods.

Diazotization with 0.1 N sodium nitrite in the presence of acid and determination of the end point with starch-iodide test paper by the recommended A. M. A. Laboratory Method[1] was investigated. The results obtained are shown in Table 1.

<div align="center">TABLE 1.—Results by A.M.A. laboratory method</div>

TAKEN	FOUND	RECOVERY
gram	*gram*	*per cent*
0.0700	0.0703	100.43
0.0700	0.0701	100.14
0.2000	0.1985	99.25
0.2000	0.1992	99.60
0.5000	0.4955	99.10
0.5000	0.4962	99.24
0.7000	0.6930	99.00
0.7000	0.6922	98.89

The method is simple and accurate, and the results are in satisfactory agreement. The objections are that the method is not specific, the sodium nitrite solution must be very frequently standardized, the reaction is slow towards the end, and experience is necessary to properly interpret the end point.

[1] *J. Am. Med. Assoc.*, **109**, 359 (1937).
[*] *Quart. J. Pharm. Pharmacol.* **9**, 560 (1936).
[2] *J. Biol. Chem.*, **122**, 263 (1937).
[3] *Lancet*, **232**, 194 (1937).
[4] *Ibid.*, 195.
[5] *Z. Anal. Chem.*, **108**, 396 (1937).
[6] Minutes of the Combined Contact Committee Meeting of March 28–29, 1938. Item No. 12.

Diazotization and coupling with dimethyl-α-naphthylamine in acid solution to produce a purple-red dye and measurement of the intensity of the color by means of comparison with a standard, as in the excellent micro method of E. K. Marshall, Jr.,[2] was used quite extensively in this laboratory for the determination of the drug in a liquid preparation, and satisfactory results were obtained (Table 2). A similar method* except that α-naphthylamine was used as the coupling agent was found to be less satisfactory. The use of β-naphthol in alkaline solution as the coupling agent according to the method of Fuller[3] was tried, and generally unsatisfactory results were obtained. The colorimetric methods are essentially micro methods, and for that purpose are excellent, especially the Marshall method. As general routine methods they are impracticable in that special organic reagents and apparatus to protect the reagents from the air and light are required, a blank determination on a "known" must be run concurrently with the sample, and finally a precision colorimeter is necessary. The overall accuracy of the Marshall method appears to be ±2 per cent.

TABLE 2.—*Results by Marshall method*

TAKEN (BEFORE DILUTION)	COLORIMETER READINGS	FOUND (CALCULATED)	RECOVERY
gram	*gram*	*per cent*	*per cent*
0.5	10.1	0.505	101
0.5	10.2	0.510	102
1.0	9.9	0.99	99
1.0	9.8	0.98	98

Mercuration with mercuric nitrate in slightly acid solution[4] was investigated. The resultant complex insoluble precipitate was dried and weighed, and although it was found possible to control the composition of the precipitate by following an exact procedure, it was necessary to assign an empirical factor to convert the weight of the precipitate to sulfanilamide. If necessary, the sulfanilamide can be recovered from the complex by precipitating the mercury with hydrogen sulfide, filtering off the mercuric sulfide, and evaporating the filtrate at about 70° C. with the aid of a fan. The residue, impure sulfanilamide, is then recrystallized from hot alcohol. Recoveries are not quantitative.

The direct bromination of the nucleus by a method similar to that used for the determination of acetanilid in *Methods of Analysis, A.O.A.C.,* 1935, appeared theoretically practical for sulfanilamide. Investigation, however, disclosed that low results, Table 3, were obtained, the formation of the dibromo derivative proceeding quite slowly towards the end.

Attention was next directed to an indirect bromination, namely that of adding an excess of a 0.1 *N* potassium bromide-bromate solution, acidifying, allowing to stand, then adding potassium iodide, and finally titrating the liberated iodine with 0.1 *N* sodium thiosulfate, using starch

* *Quart. J. Pharm. Pharmacol.* 9, 560 (1936).

as indicator. Two papers proposing this method have since appeared in the literature.[5,6] Preliminary work on this method consisted of ascertaining the effect of the several factors that might influence the accuracy of the method. These factors and the results obtained when they were varied are shown in Table 4. Unless otherwise stated the sample consisted in all cases of 0.15 gram of recrystallized sulfanilamide.

TABLE 3.—*Results by A.O.A.C. acetanilid method*

TAKEN	FOUND	RECOVERY
gram	*gram*	*per cent*
0.2000	0.1948	97.4
0.2000	0.1930	96.5
0.5000	0.4885	97.7
0.5000	0.4877	97.5

TABLE 4.—*Factors influencing indirect bromination method*

EFFECT OF VARYING THE EXCESS OF BROMINE PRESENT

0.1 N BROMINE PRESENT	0.1 N BROMINE REQUIRED	EXCESS BROMINE PRESENT	SULFANILAMIDE
cc.	*cc.*	*per cent*	*per cent*
34.85 (theory)	34.54	1	99.13
38.86	34.96	11.2	100.34
48.58	35.02	28.7	100.51
58.29	35.06	66.3	100.62
97.15	35.54	173	102.00

EFFECT OF TIME OF BROMINATION
50.00 cc. 0.1 N bromine added

TIME OF STANDING	SULFANILAMIDE
	per cent
About ½ min.	100.39
5 min.	100.51
15 min.	100.47
45 min.	100.51
12 hours	104.58

EFFECT OF VOLUME OF LIQUID DURING BROMINATION

LIQUID PRESENT		TOTAL VOLUME	SULFANILAMIDE
cc.		*cc.*	*per cent*
10 cc. HCl+10	0.5 N Br	20	100.82
+50	0.1 N Br	60	100.93
25 cc. H₂O+10	HCl		
+50	0.1 N Br	85	101.20
65 cc. H₂O+10	HCl		
+50	0.1 N Br	125	102.40

EFFECT OF LIGHT ON BROMINATION

CONDITION DURING BROMINATION—	SULFANILAMIDE FOUND	SULFANILAMIDE RECOVERY
	gram	*per cent*
In dark	0.1507	100.48
Diffused daylight	0.1508	100.56
Bright sunlight	0.1513	100.85

EFFECT OF TEMPERATURE ON BROMINATION

TEMPERATURE DURING BROMINATION	SULFANILAMIDE FOUND	SULFANILAMIDE RECOVERY
	gram	*per cent*
Room temp. (24° C.)	0.1507	100.47
Room temp. (24° C.)	0.1508	100.53
Refrigerator (8° C.)	0.1504	100.27
Refrigerator (8° C.)	0.1503	100.20
Ice-water (1.5° C.)	0.1503	100.20
Ice-water (1.5° C.)	0.1502	100.13

A comparison of the preliminary results obtained shows that under similar conditions check results were obtained and were reproducible. Within normal working limits none of the factors investigated appreciably affected the results obtained. In general the results are above the theoretical, and this was first attributed to the almost inevitable loss of small amounts of bromine when the potassium iodide solution was introduced. Later work indicated that the excess found was probably due to a retention of bromine by the formation of a sulfondibromamide with the sulfanilamide. It may also be noted that a difference of 0.1 cc. of 0.1 N thiosulfate in the titration made a difference of ± 0.3 per cent in the per cent recovery figure.

Despite the fact that the indirect bromination method yielded somewhat high results, it is believed that it should be collaboratively studied, especially as it was more accurate than the colorimetric methods, which were the only other methods available when this work was begun. The following collaborative samples were prepared:

Sample A.—Consisted of 7.3 grain tablets of sulfanilamide prepared commercially under the supervision of the Associate Referee. The tablet mixture contained 69.5% and the dried granulations contained 68.4% of sulfanilamide, the balance being composed of starch and talc. The actual sulfanilamide content of the tablets as determined from the Kjeldahl nitrogen content was 68.6 per cent. 5000 of the tablets were aged and dusted, and portions were placed in clean containers for the collaborators.

Sample B.—Purified sulfanilamide (see first page this report).

Sample C.—Consisted of a powder composed of the ingredients listed on p. 753.

Nitrogen was determined by the Kjeldahl method on the mixture and also on the gelatin component. After due correction for gelatin nitrogen, the nitrogen was equivalent to 47.48 per cent sulfanilamide.

	per cent
Sulfanilamide	47.37
Talc	11.70
Gelatin	2.92
Lactose	5.85
Chalk	5.85
Gum acacia	2.92
Calcium phosphate	5.85
Potato starch	11.70
Kaolin	5.85
Total	100.01

Each component was ground to pass a 60-mesh sieve, then weighed out and mixed. The mixture was psssed through a 60-mesh sieve three times, subdivided by quartering and requartering, then placed in clean containers and sealed.

Collaborators were supplied with portions of each of the above samples and were requested to make duplicate determinations by Method 1 as follows:

METHOD I

Place a portion of the sample containing 0.1–0.3 gram of sulfanilamide, in a 500 cc. glass-stoppered iodine absorption flask,* add about 25 cc. of water and sufficient standard bromide-bromate solution (0.1 N or 0.5 N) (0.5 N bromide-bromate solution, *Methods of Analysis*, A.O.A.C., 1935, 543. 0.1 N bromide-bromate solution, *Ibid.*, 551) to ensure a 10 to 50 per cent excess of Br. Add rapidly 10 cc. of HCl and immediately insert the stopper. Swirl the flask, and place in the dark about 5 minutes. Remove the stopper just sufficiently to introduce quickly 10 cc. of 10 per cent KI solution, taking care that no Br vapors escape, and immediately stopper the flask. Shake thoroughly, remove the stopper, and rinse it and the neck of the flask, receiving the washings in the flask. Add about 150 cc. water, and then titrate the liberated I with 0.1 N $Na_2S_2O_3$ solution, using starch indicator.

1 cc. of 0.1 N bromide-bromate solution = 0.004305 gram of sulfanilamide.

NOTES

(1) If carbonates are present, make the sample slightly acid and allow the CO_2 to pass off; then make slightly alkaline with NaOH (1+10), and proceed as directed previously.

(2) If difficulty is experienced by loss of Br vapors, cool the stoppered flask before the addition of the KI solution.

The collaborators' results are reported in Table 6.

COLLABORATORS

Charles F. Bruening, U. S. Food and Drug Adm., Baltimore.
Donald C. Grove, U. S. Food and Drug Adm., Washington.
Maurice Harris, U. S. Food and Drug Adm., Houston.
The Associate Referee.
William F. Reindollar, State Department of Health, Baltimore.
S. Reznek, U. S. Food and Drug Adm., New York City.
Phileas A. Racicot, Dept. of Public Health, Food and Drug Div., Boston.

* The use of an iodine absorption flask was not specified in the method sent to the collaborators.

TABLE 6.—*Collaborative results*

COLLABORATOR	SULFANILAMIDE		
	SAMPLE A	SAMPLE B	SAMPLE C
	per cent	*per cent*	*per cent*
1	70.71	101.9	49.91
	70.77	101.9	49.84
2	69.72	100.24	48.96
	69.83	100.39	48.96
3	70.68	100.86	49.36
	70.44	100.90	49.06
4	69.88	100.65	48.52
	69.90	100.51	48.86
5	68.42	99.26	48.29
	69.02	99.55	47.70
6	70.6	100.0	48.6
	70.9	100.3	48.6
7	73.0	101.4	55.9
	73.4	100.6	55.1

COMMENTS OF COLLABORATORS (ABSTRACTED)

Bruening.—Special iodometric flasks were used, so designed that the 10 cc. of KI solution can be placed in the reservoir above the stopper and as the stopper is lifted the KI solution is drawn into the flask and no bromine vapor is lost. (Also commented on high results.)

Grove.—Suggested iodine flasks and commented on high results obtained.

Harris.—It is noted that the brominated sulfanilamide absorbed iodine during the back titration with thiosulfate, as evident by the absence of a pure white precipitate. If a small amount of alcohol is added near the end point, it will displace the iodine from the surface. The high results indicated for Sample B may be due to undesired substitution and can be avoided by brominating at low range temperatures as 0°–5° C. The selected temperature should also insure quantitative substitution. (An iodine absorption flask was also suggested.)

Reindollar.—The blue color tends to return after titration flasks have stood awhile. This does not interfere. During the 5 minute reaction period the flasks were placed in ice water . . . ."

Reznek.—Noted slightly high results.

Racicot.—Suggested that a cork-stoppered Erlenmeyer flask provided with dropping funnel with cock be used to prevent loss of bromine vapor.

DISCUSSION

It is evident from the results of the collaborators, Table 6, that the method yields somewhat high results in the case of all three samples. Investigation of several factors that might be conducive to the production of such excesses has disclosed no points whereby the method can be modified so that quantitative yields might be obtained. As previously stated the excess is apparently due to a reaction of the bromine with the sulfonamido group and the formation of a sulfondibromamide, the bromine of which is not wholly liberated under the conditions of the test.

<div align="center">

RECOMMENDATIONS[1]

</div>

It is recommended—

(1) That the method described in this report be adopted as an alternative tentative method (see Part II).

(2) That due notice be made of the tendency of the method to yield slightly high results.

<div align="center">

PART II

EXPERIMENTAL

</div>

It is generally agreed that the sulfonamido group ($-SO_2NH_2$) is the therapeutically important group in sulfanilamide and its many derivatives, which are being made commercially available. It would appear that a method based on the determination of this group would be more desirable and certainly more specific than any of the methods discussed in Part I.

This group is, in general, resistant to reduction and to hydrolysis, and due to its stability does not enter into many reactions with ordinary reagents. In this connection the work of Chattaway[2] was reviewed. This worker found that some sulfonamides react quantitatively with hypobromous acid and form relatively stable sulfondibromamides. These compounds are soluble in organic reagents and on reacting with strong acids liberate the bromine, which then can be determined iodometrically. The fact that this method has failed to yield quantitative results to date when applied to sulfanilamide may be explained by the fact that Chattaway used benzenesulfonamide and toluenesulfonamide instead of the amino derivative. Sulfanilamide reacts with the hypobromous acid in a vigorous manner, and unless cooled, oxidation and even carbonization may occur. With suitable precautions it was found possible to prepare the sulfondibromamide, extract, and determine the amount present by iodometric titration. Poor yields were obtained. In an effort to increase the yield the sulfanilamide was acetylated and the acetyl derivative was then treated by the following outlined method:

A 0.25 gram sample of sulfanilamide was acetylated in a separator by adding an excess of acetic anhydride, shaking vigorously, and allowing to stand until clumps of needle-like crystals separated. The separator and its contents were cooled to about 2° C., and 25 cc. of a solution of hypobromous acid* cooled to 2° C. was added. The separator was shaken for 5 minutes and frequently immersed in ice water. The sulfondibromamide was then extracted with several portions of chloroform, each portion being washed with 5 cc. of the hypobromous acid solution. The combined chloroform extracts were filtered through cotton and evaporated at room temperature with the aid of a fan. The residue was dried over sulfuric acid and weighed as the dibrom derivative. It may be taken up in dilute alkali, transferred

[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 58 (1939).
[2] *J. Chem. Soc.*, 87, 145 (1905).
* Prepared by shaking Br with precipitated HgO suspended in water.

to an iodine absorption flask, the bromine determined iodometrically by the addition of potassium iodide, acid, and water, and the liberated iodine titrated with thiosulfate, starch being used as indicator.

By the use of this method it was possible to obtain yields up to 80 per cent of the sulfanilamide added. Due to the numerous steps in this method, the care that must be exercised in handling the sulfondibromamide, and the poor yields obtained, no further work was done on this method. However it is contemplated that further work will be done along these lines, since in addition to being useful to separate the sulfanilamide from other drugs the method is quite specific for sulfanilamide and many of its derivatives.

A somewhat simpler method, which could be applied to the analysis of the sulfonamido group, is that of hydrolysis, whereby the ammonium sulfate formed when sulfuric acid is used as the hydrolyzing agent is determined by making alkaline, distilling, and determining the ammonia in the distillate. Preliminary work on this method was confined to determination of optimum conditions for hydrolysis of the sulfanilamide, and the results are embodied in Method II.

METHOD II

Place on a 9 cm. folded filter paper in a funnel a portion of the sample containing about 0.5 gram of sulfanilamide. Wash the soluble portion with a fine stream of acetone into a 250 cc. flask, using a total of about 25 cc. of acetone, Test for complete extraction by evaporating a small portion of the washings. Immerse the flask in a water bath at about 70° C. until the acetone has been evaporated and its odor is no longer perceptible. Remove from bath and add 10–12 cc. of 75% (by volume) H_2SO_4. Connect the flask to a reflux condenser with water jacket, add a few glass beads, and boil slowly for 30 minutes. Wash down the condenser with water, make the liquid in the flask to about 100 cc. with water, add an excess of 50% alkali, distil, and collect the ammonia in the distillate in an excess of 0.1 N H_2SO_4. Titrate the excess acid with 0.1 N NaOH, using methyl red indicator.

1 cc. of 0.1 N $H_2SO_4 = 0.01722$ gram of $(NH_3)_2C_6H_8SO_2$.

After the preliminary work on this method had been carried out, and a short time before the 1938 A.O.A.C. Meeting, a few collaborators were requested to make duplicate determinations on Samples A, B, and C by Method II. In spite of the last-minute request four of the collaborators very kindly responded. Their collaborative results appear in Table 7.

The collaborators were Charles F. Bruening; Llwellyn H. Welsh, U. S. Food and Drug Adm. Baltimore; William F. Reindollar, and the Associate Referee.

COMMENTS OF COLLABORATORS (ABSTRACTED)

Bruening.—The end point was somewhat difficult to ascertain; the distillates were titrated until practically all the red color disappeared.

Welsh.—Do not believe necessary to keep temperature below 100° C. in removal of acetone.

Reindollar.—In spite of the approximate results obtained (due to interruptions

during analysis), I feel that with due attention and experience the method is capable of yielding better results, and is certainly more specific than the bromination method.

TABLE 7.—*Collaborative results by Method II*

COLLABORATOR	SULFANILAMIDE		
	SAMPLE A	SAMPLE B	SAMPLE C
	per cent	per cent	per cent
1	69.1	100.0	48.0
	69.5	100.2	47.9
2	69.11	99.88	47.87
	68.63	100.08	47.96
3	68.71	99.98	47.70
	68.86	100.01	47.53
4	67.91	99.35	48.05
5			
Averages:	68.83	99.93	47.86

DISCUSSION

The results obtained by Method II, Table 7, indicate a more satisfactory agreement with the known sulfanilamide content of the collaborative samples than do the results obtained by Method I, Table 6. In the second method the results are slightly high, although the average appears to be within the analytical error for a determination of this nature.

Method II is simple and short and requires reagents and apparatus found in all analytical laboratories. It has received no fundamental adverse criticism from collaborators.

Due to the late date at which the method was developed, it has been studied by only four collaborators. In spite of this fact the Associate Referee believes that no additional work is necessary, first because the method is direct, and secondly because it is believed that this drug will be included in the forthcoming U.S.P. XI Supplement No. 2, which will remove it from the scope of the A.O.A.C. unless no assay method is provided by the U.S.P. XI.

It is recommended,[1] therefore, that the method described in Part II of this report be adopted as a tentative method.

REPORT ON MANDELIC ACID

By H. G. UNDERWOOD (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The study of mandelic acid was undertaken in accordance with the recommendation of Subcommittee B. It appeared to be desirable to investigate the properties of mandelic acid and to develop a method for

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[1] For report of Subcommittee B and action by the Association, see *This Journal*, 22, 58 (1939).

its determination in typical market preparations. This product and its salts have been used recently for the treatment of infections of the urinary tract.

The results of collaborative study and recommendations are included in this report.

REVIEW OF LITERATURE

Mandelic acid, $C_6H_5CH(OH)COOH$, due to the presence of an asymmetric carbon atom in the molecule, exists in three definite forms. The synthetically prepared compound is the racemic or (dl) mandelic acid, and hence is optically inactive. Mandelic acid is a white, crystalline compound, which gives colorless and clear solutions in water, alcohol, and alkali. Its melting point has been reported by Claisen[1] to be 118° C. and by others to be 118–120° C.[2] The solubility is 15.95 grams at 20° C. in 100 cc. of water; 53.6 grams dissolve in 100 cc. ethyl alcohol at 16.5° C. It is also soluble in ether and other organic solvents. The pure acid is slowly decomposed by light, with the liberation of benzaldehyde, and should, therefore, be stored in the dark, or in suitable light-proof bottles. Mandelic acid reacts readily with basic substances to produce salts or "mandelates."

The sodium, ammonium, calcium, and magnesium salts as well as compounds of mandelic acid with ethanolamine, ethylene diamine, and hexamethylenetetramine have been reported of value in mandelic acid therapy. In general, the drug is encountered in tablet form and solutions, usually as a mandelate.

EXPERIMENTAL

Several qualitative tests for mandelic acid are reported in the literature. An aqueous solution of mandelic acid gives the test for an alpha-hydroxy acid with ferric chloride.[2] Mandelic acid when treated with sulfuric acid, under certain conditions of concentration, gives a purple coloration and the odor of benzaldehyde.[3] The Associate Referee requested the collaborators to report on these tests to determine whether they are suitable for the identification of mandelic acid. The collaborators were also requested to report on the melting point by the U.S.P. method. The mandelic acid submitted for the above tests titrated 99.9 per cent.

F. Reimers[4] reports that mandelic is quantitatively isolated from hydrochloric acid solution by 5 or 6 extractions with a double volume of ether. The ether is evaporated on a water bath and the residue titrated with 0.1 N sodium hydroxide, phenolphthalein being used as indicator. E. M. Hoshall[5] of the Baltimore Station, U. S. Food and Drug Administration, also reports practically quantitative recovery by ether extraction and titration.

[1] *Ber.*, 10, 847 (1877).
[2] *J. Am. Med. Assoc.*, 109, 24, 1989 (1937).
[3] Mulliken, Vol. 1.
[4] *Dansk Tids Farm.*, 12, 25–32 (1938).
[5] Private communication.

Collaborative results on mandelic acid

COLLABORATOR	SAMPLE 1 TABLET METHOD		ELIXIR TITRATION WITH 0.5 N NaOH		ELIXIR DILUTION METHOD TITRATION WITH 0.1 N NaOH		M. P. MANDELIC ACID U. S. P. METHOD
	g./100 cc.	% RECOVERY	g./100 cc.	% RECOVERY	g./100 cc.	% RECOVERY	
Theoretical	90.0		40.0		40.0		
I. S. Shupe Kansas City	90.0 89.9	100.0 99.9	39.8 39.6	99.5 99.0			118.5–119° C.[1]
J. Carol Cincinnati	89.6 89.6	99.6 99.6	39.9 40.0 39.7	99.8 100.0 99.3			116 –118° C.
R. Hyatt Cincinnati	89.85 89.85	99.8 99.8	39.9 40.0	99.8 100.0	40.0 40.0	100.0 100.0	118 –119° C.
C. B. Stone Cincinnati	89.6 89.6	99.6 99.6	40.05 40.05	100.1 100.1	40.0 40.1	100.0 100.3	
L. D. Seif* Cincinnati (N. C. Anewalt, Analyst)	87.43 88.19	97.1 98.0			39.4 39.0	98.5 97.5	118 –119° C.
G. M. Johnson Minneapolis	89.71 89.82 89.79 89.71	99.7 99.8 99.8 99.7	39.67 39.63 39.70 39.79	99.2 99.1 99.3 99.5			
H. G. Underwood Chicago	89.9 90.0 89.7	99.9 100.0 99.7	39.84 39.84	99.6 99.6	40.0 40.0	100.0 100.0	118 –119° C.
Average, all values	89.54	99.5	39.83	99.59	39.81	99.54	

* Wm. S. Merrell Co., all other collaborators U. S. Food & Drug Adm.
[1] On the same thermometer benzoic acid melted at 121.5–122° C.

The Associate Referee tried a mixed solvent of chloroform and ether (2+1) and found it satisfactory for the extraction of mandelic acid from solution. The chloroform-ether solvent, in contrast to ether, makes for more convenient manipulation, since it forms the lower layer in the separator. Since mandelic acid titrates readily, no attempts were made to determine it gravimetrically. Furthermore, mandelic acid sublimes slowly at 100° C., and any gravimetric method would necessitate drying at a lower temperature or in a desiccator.

Two samples were prepared and submitted to collaborators with the proposed methods. The first consisted of a mixture of mandelic acid (90 per cent) and starch. The second sample, an elixir, was prepared to represent a liquid preparation and was compounded as follows:

Mandelic acid	400 grams
NH₄OH solution to neutral.	
Tincture sweet orange peel	20 cc.
Sirup	100 cc.
Alcohol	200 cc.
Water to make 1000 cc.	

The methods were published in *This Journal*, **22**, 98 (1939). The results are given in the table.

COMMENTS BY COLLABORATORS

I. S. Shupe.—Tests on both methods showed incomplete extraction with the seventh portion of solvent. The eighth and ninth extractions still showed traces of mandelic acid, but the titrations were insignificant. The qualitative tests responded as described in your method. The qualitative color test with sulfuric acid did not respond when too small an amount of mandelic acid was used.

G. M. Johnson.—(a) A bright yellow color was obtained. (b) A purple tint was obtained. Neither of the above seems to be a very conclusive test.

L. D. Seif.—The qualitative tests were satisfactory, although the colors produced were not very intense. The bright yellow color produced with FeCl₃ was slightly more intense than that produced by adding the same amount of FeCl₃ to distilled water. We have tried a qualitative test by melting mandelic acid and hydroquinone together and dissolving in 10 per cent NaOH. The color produced here was no more definite than the color produced in the other tests.

R. Hyatt.—The qualitative tests were observed to give the results indicated in the description. In (b) the development of the purple color was slow if the solutions were mixed. If the solutions were not mixed, the color development was quicker and just as characteristic. The tests appear to be satisfactory.

C. B. Stone.—The qualitative tests gave the results described in the methods. If too small a sample was used or if the solutions were rapidly mixed, the purple color did not form in the test with H₂SO₄. However, in both cases, the odor of benzaldehyde was noted.

DISCUSSION

The results obtained by the collaborators on the two samples are quite satisfactory. About seven extractions are necessary to remove the mandelic acid, this number being necessitated, no doubt, by the relatively high solubility of mandelic acid in water.

The average value for the melting point of mandelic acid as determined by the U.S.P. method is 118°–119° C. and is in the range of the values reported in the literature.

The collaborators found that the qualitative tests responded as described in the method if the directions were carefully followed. The Associate Referee and several of the collaborators observed that the qualitative color tests with sulfuric acid did not respond or that the purple color developed very slowly if too small an amount of mandelic acid was used; however, the odor of benzaldehyde was always noted. While the qualitative tests are none too sensitive, the Associate Referee believes that if they are used in conjunction with the melting point they will be of service in the identification of mandelic acid.

RECOMMENDATIONS[1]

It is recommended—

(1) That the qualitative tests be adopted as tentative.

(2) That the methods for the determination of mandelic acid in tablet and liquid preparations be adopted as tentative.

REPORT ON CHLOROFORM IN MIXTURES

By John R. Matchett (Treasury Department Laboratories,
Washington, D. C.), *Associate Referee*

The present (tentative) method for the determination of chloroform in mixtures is due to Roberts and Murray[2] and to experimental studies carried on during 1930 by Matchett, *This Journal*, 14, 360 (1931).

The method was published in *Methods of Analysis*, A.O.A.C., 1935, 579.

During the present year the following criticisms and suggestions have been made by A.O.A.C. members and others:

1. Reagent 106(a), saturated KOH in methyl alcohol, might well be substituted for reagent 104(a), thus eliminating one reagent. The reagent proposed is necessary for hydrolysis of CCl_4.

2. The alkali solution at present specified is more concentrated than need be.

3. The term "citrate bottle" should be changed to "pressure bottle" and the words "fitted with a rubber gasket to provide a tight seal" added.

4. The directions should include the following words of caution with regard to the handling of pressure bottles: "Caution: Do not cool pressure bottle suddenly. It is best to allow it to cool in the H_2O in which it was boiled."

5. The amount of $CaCO_3$ added to the sample prior to distillation should be reduced from 1 gram to 0.1 gram.

6. Previous work by Associate Referee Matchett was inadequate, especially in that only three analyses indicating that a suitable recovery of $CHCl_3$ was possible were reported.

DISCUSSION

The criticisms and suggestions are well taken. No objection can be offered to the adoption of the changes proposed under 1, 3, 4, and 5. It is probably true also that less concentrated alkali would serve equally well for the hydrolysis of the chloroform. The advantage of utilizing a single reagent for both chloroform and carbon tetrachloride, however, outweighs this consideration, especially since no particular disadvantage accrues from its use.

During the previous scrutiny of the method it developed, late in the season, that collaborative results, although reproducible as indicated by check analyses, were decidedly too low. Approximately 0.5 gram of chloroform per 100 cc. was found in samples to which approximately 0.9 gram per 100 cc. had been added. These findings led to the presumption that chloroform had been lost by evaporation, or otherwise, and experiments that corroborated this view were carried out.

It appeared advisable then to add known amounts of chloroform to individual samples immediately prior to distillation. This was done and the three recorded results obtained.

The recovery in these analyses, though not gratifying, appeared adequate for the purpose and no reason whatever was seen to indicate the results could not be repeated. It is true that no possible interfering substances, especially plant extractives, were present. The method, however, is prescribed, not for a specific type of sample but for chloroform in mixtures. Any mixture of unknown composition must be examined to insure the absence of interfering substances before being assayed by any method for any constituent, hence nothing appeared to be gained by adding extractive matter from any drug.

In view of the foregoing, the evident reproducibility of results, and the analyses recorded by Roberts and Murray, the Associate Referee recommended that the method be adopted as tentative. The recommendation was accepted and the subject closed at that time.

The editorial review of *Methods of Analysis*, requested by the General Referee on Drugs, made it desirable to further examine the method in the light of the criticisms and suggestions offered. To this end the analytical results summarized in Tables 1, 2, 3, and 4 are offered.

The following procedure was followed:

Table 1.—Samples containing a known weight of $CHCl_3$ (labeled c.p., containing approximately 0.5% alcohol) dissolved in 10 cc., of alcohol were pipetted into pressure bottles containing 25 cc. of saturated methyl alcoholic KOH and 60 cc. of alcohol. The bottles were sealed, and the analysis was carried on in the specified manner. Results indicated that the $CHCl_3$ used was 99.1% pure.

Table 2.—To 20 cc. portions of a sirup containing 625 grams of sugar and 40 grams of NH_4Cl per liter were added 65 cc. of alcohol and 10 cc. of an alcoholic solution containing a known amount of $CHCl_3$. The samples were distilled and analyzed as prescribed, alkaline reagent 106(a) and 0.1 gram of $CaCO_3$ being used. Taking 99.1% of the weighed amount of $CHCl_3$ as the actual amount present, the average recovery in these analyses was 98.9%.

Table 3.—20 cc. samples of compound Sirup of White Pine, N.F. VI, stated to contain 3 minims of $CHCl_3$ per fluid ounce, were analyzed, and the results were recorded in Analyses 1, 2, and 3. To similar samples were added known amounts of $CHCl_3$ and the analysis was carried on in the same way at the same time. The results are shown in Analyses 4 and 5. The two analyses gave practically identical results with indicated recovery of 98.2% based on the $CHCl_3$ present (average of 1, 2, and 3) and 99.1% of the $CHCl_3$ weighed and added.

Table 4.—Two 200 cc. samples were prepared containing identical amounts of $CHCl_3$. In sample "A" the menstruum was the sirup referred to in Table 2, plus 10% alcohol; in sample "B" the menstruum was alcohol. Analytical samples were pipetted from each. The low recovery recorded under samples "A" indicates the necessity for exercise of extreme care in sampling.

TABLE 1.—*Samples not distilled*

	CHCl₃ ADDED	CHCl₃ FOUND	RECOVERY
	gram	*gram*	*per cent*
1	.1470	.1455	98.9
2	.1470	.1457	99.1
3	.1476	.1466	99.3
4	.1476	.1462	99.0
5	.1476	.1465	99.2
6	.1469	.1454	99.0
7	.1469	.1461	99.4
		Av.	99.1

TABLE 2.—*Samples distilled as directed from sirup containing 625 grams of sugar and 40 grams of NH_4Cl per liter*

	CHCl₃ ADDED	ACTUAL CHCl₃ ADDED*	CHCl₃ FOUND	RECOVERY
	gram	*gram*	*gram*	*per cent*
1	.1476	.1463	.1456	99.5
2	.1476	.1463	.1443	98.6
3	.1476	.1463	.1436	98.1
4	.1476	.1463	.1466	100.2
5	.1476	.1463	.1430	97.8
6	.1476	.1463	.1440	98.4
			Av.	98.8

* 99.1% of weight of added $CHCl_3$.

TABLE 3.—*Samples of compound sirup of white pine to Nos. 4 and 5 of which were added weighed amounts of $CHCl_3$ and the analyses conducted in manner prescribed*

	CHCl₃ PRESENT	CHCl₃ ADDED	CHCl₃ FOUND	RECOVERY
	gram	*gram*	*gram*	*per cent*
1		None	.0940	
2		None	.0933	
3		None	.0930	
4	.0934[1]	.0729[2]	.1650	98.2[3]
5	.0934[1]	.0729[2]	.1651	98.2[3]

[1] Average of Determinations 1, 2, and 3.
[2] 99.1% of $CHCl_3$ weighed.
[3] Based on difference between $CHCl_3$ found and average of $CHCl_3$ found in Analyses 1, 2, and 3.

TABLE 4.—*Samples A pipetted from sirup referred to in Table 2, plus 10% alcohol. Samples B pipetted from alcohol like those reported in Table 2. Analysis same as in Tables 2 and 3.*

	$CHCl_3$ ADDED	ACTUAL $CHCl_3$ ADDED*	$CHCl_3$ FOUND	RECOVERY
	gram	*gram*	*gram*	*per cent*
A	.1469	.1456	.1395	95.8
A	.1469	.1456	.1395	95.8
B	.1469	.1456	.1437	98.7
B	.1469	.1456	.1441	99.0

* 99.1% of weight of added $CHCl_3$.

OBSERVATIONS AND CONCLUSIONS

1. The use of reagent 106(a) is satisfactory.
2. Use of 0.1 gram of calcium carbonate is satisfactory.
3. Carborundum chips are very useful to prevent "bumping" during distillation.
4. The method may be relied upon to show at least 98 per cent recovery of chloroform present in the sample.
5. As much as 5 per cent of the chloroform present may be lost on pipetting a sample of heavy sirup.
6. By use of special apparatus recovery might be increased and more concordant results obtained.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method be amended to reduce the calcium carbonate from 1.0 to 0.1 gram.

(2) That the method be amended to permit the use of carborundum chips to prevent bumping.

(3) That the term "citrate bottle" be deleted and the words "pressure bottle" be substituted.

(4) That the following caution be inserted in an appropriate place:

Caution: Do not cool the pressure bottle suddenly. It is best to allow it to cool in the water in which it was boiled.

(5) That the method (after amendments) be retained in its tentative status.

The analyses reported herein were made by Louis Benjamin, Joseph Levine, and G. F. Beyer, to whom the Associate Referee makes grateful acknowledgment.

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[1] For report of Subcommittee B and action by the Association, see *This Journal,* 22, 58 (1939).

CONTRIBUTED PAPERS

A RAPID METHOD FOR CHLORIDES IN TOMATO PRODUCTS

By L. M. BEACHAM (U. S. Food and Drug Administration, Washington, D. C.)

The present official method[1] for the determination of chlorides in tomato products requires drying on a steam bath and ashing, which makes the method slow and time-consuming. The tentative method[2] for chlorides in tomato juice, which it has been suggested might be used for other tomato products as well, requires filtering the product. Tomato products tend to clog the filter, and filtration is difficult. The following method has been found to obviate these difficulties and to give excellent results:

METHOD

Weigh 5 grams of the tomato material and transfer with 80% C_2H_5OH to a 100 cc. volumetric flask. Then add the C_2H_5OH to give a volume of approximately 50 cc. Shake well to get all the tomato material into suspension. Add 1 cc. of concentrated HNO_3 and by means of a pipet add 25 cc. of 0.1 N $AgNO_3$. Make to 100 cc. volume with alcohol, transfer to a centrifuge bottle, and centrifuge at 1800 r.p.m. for 5 minutes. Pipet 50 cc. of the supernatant liquid into a 300 cc. Erlenmeyer flask, add 2 cc. of a saturated solution of $FeNH_4(SO_4)_2$ and titrate to a permanent light brown color with 0.1 N NH_4CNS solution. Multiply the number of cc. of NH_4CNS used by 2 and subtract from 25. Multiply the difference by .005843 to obtain the weight of chlorides present expressed as grams of NaCl. Divide by 5 and multiply by 100 to calculate the percentage of salt present.

Analyses were made on a number of samples of tomato products by both the official method and the one described. Some typical results are given in Table 1.

TABLE 1.—*Typical results (NaCl) on tomato products*

PRODUCT	OFFICIAL METHOD	ALCOHOL EXTRACTION METHOD
	per cent	*per cent*
Paste	1.31	1.32
	1.32	1.32
Paste	2.14	2.13
	2.14	2.10
Paste	2.13	2.13
	2.13	2.13

Authentic samples of tomato paste and tomato juice were prepared and analyzed by the alcohol extraction method for salt naturally present. Known amounts of salt were also added to subdivisions of these authentic

[1] *Methods of Analysis*, A.O.A.C., 1935, 500, 22.
[2] *This Journal*, 20, 78 (1937).

samples, and analyses were made by the alcohol extraction method to determine the total salt present. The results are given in Table 2.

TABLE 2.—*Salt naturally present and added*

PRODUCT	SALT ADDED	NATURAL SALT	TOTAL SALT BY CALCULATION	TOTAL SALT BY ALCOHOL EXTRACTION
	per cent	*per cent*	*per cent*	*per cent*
Paste	2.00	.314	2.314	2.320
				2.320
Juice	1.00	.042	1.042	1.047
				1.047

SUMMARY

A rapid method for determining the chlorides present in tomato products by means of alcoholic extraction and precipitation with silver nitrate is described. When compared with the present official method, the method gives equally accurate results, as it does when used on authentic samples having a known amount of chlorides added.

AN OBJECTIVE METHOD FOR MEASURING GRITTINESS IN CANNED PEARS

By L. M. BEACHAM (U. S. Food and Drug Administration, Washington, D. C.)

Grittiness is one of the varietal characteristics of canned pears. For example, pears of Kieffer variety have a high degree of grittiness, while Bartlett pears are relatively free from it. Investigation indicates that the grittiness is caused by the presence of grit cells approximately 0.02 inch or more in diameter. Pears containing only cells of smaller diameter, or even these smaller cells alone, may be chewed and swallowed without any impression of the presence of hard material.

The following method was developed for determining in canned pears the amount of grit cells retained on a 30-mesh screen (that is, of approximately 0.02 inch diameter):

METHOD

Drain the pears, with the "cups" down, for 2 minutes on an 8-mesh screen with wire diameter of .013 inch, using an 8-inch screen ("Standard Specifications for Sieves," U. S. Department of Commerce, National Bureau of Standards Bulletin of October 25, 1938) for containers of less than 3 pounds net weight, and a 12-inch screen for larger containers. Remove the pears to a food chopper and grind until homogeneous. Place 50 grams of the ground material in the cup of a malted milk stirrer (a Hamilton Beach Model 25 was used in these experiments), and add 200 cc. of water and 25 cc. of 50% NaOH solution. Bring the mixture to a boil and boil vigorously for 5 minutes; place under the stirrer and stir for 5 minutes; and filter

through a tared 30-mesh Monel metal wire screen ("Standard Specifications for Sieves," U. S. Department of Commerce, National Bureau of Standards Bulletin of October 25, 1938) fitted into a Büchner funnel. Wash with copious quantities of hot water until the grit cells are free of adhering pear material, and the wash water is free from visible cells. Dry screen and retained cells at 100° C. for 1 hour and weigh. Calculate the percentage of grit cells retained on the screen.

Samples of canned Bartlett, Kieffer, and Pineapple pears were examined. The results are listed in the table.

VARIETY	ORIGIN	TASTE	GRIT CELLS RETAINED
			per cent by weight
Bartlett	California	Smooth	.03 .02 .02
Bartlett	Oregon	Smooth	.03 .04
Bartlett	Michigan	Smooth	.04 .05 .05 .06
Bartlett	Michigan	Smooth	.02 .03
Bartlett	Oregon	Smooth	.01
Bartlett	Oregon	Smooth	.04 .06
Bartlett	New York	Smooth	.04 .04
Kieffer	Michigan	Gritty	.42 .40 .42
Kieffer	Michigan	Gritty	.48 .54
Kieffer	Michigan	Gritty	.36 .40
Pineapple	Mississippi	Gritty	.20 .19
Pineapple	Mississippi	Gritty	.27
Pineapple	Mississippi	Gritty	.31

SUMMARY

A method is described for separating and determining in canned pears grit cells retained on a 30-mesh screen.

A table shows the percentage by weight of such grit cells in samples of canned Bartlett, Kieffer, and Pineapple pears, and a comparison of the grit cells (per cent) with an organoleptic examination of such pears.

THE FREEZING POINT OF MILK

By Lincoln M. Lampert (Dairy Service Laboratory, California
State Department of Agriculture, Sacramento, Calif.)

In a paper, entitled "The Cryoscopy of Milk," Julius Hortvet[1] described the instrument now widely used for the measurement of the freezing point of milk. He reported the freezing-point depression of genuine milk to range from 0.534° to 0.562° C., with an average of 0.548° C. It would appear that the currently accepted value for the freezing-point depression for milk was obtained by rounding this figure to 0.55°. Bailey[2] presented data on collaborative work on milk from herds and normal individual cows as summarized in Table 1.

TABLE 1.—*Average freezing-point depression of authentic milk*
(From data presented by Bailey)[2]

MILK FROM INDIVIDUAL COWS	RANGE	AVERAGE
130 samples	0.523–0.580	0.546
Milk from 36 herds	0.529–0.557	0.545
(Total of 299 cows)		
Mixed milk from 2300 cows	—	0.541–0.540
Average of all values	—	0.544

Studies made in the Union of South Africa[3] gave results between 0.528° and 0.561° C., with an average of 0.541° C.

Stubbs and Elsdon[4] examined 1000 samples of milk, a few of which were obtained from individual cows, but mostly from herds. The freezing-point depressions, determined by the official A.O.A.C. method, ranged from 0.529° to 0.563°, the average being 0.544° C.

Data obtained by the Dairy Service Laboratory of the California State Department of Agriculture show that samples of pure milk often have a freezing-point depression well above 0.550° C. It was therefore deemed desirable to obtain freezing-point data on a few samples from different parts of the State. These were obtained by State Dairy Inspectors under conditions that insured that the samples were genuine and unadulter-

[1] *J. Ind. Eng. Chem.*, 13, 198 (1921).
[2] *This Journal*, 5, 484 (1922).
[3] *Analyst*, 62, 44 (1937).
[4] *Ibid.*, 59, 146 (1934)

ated. The samples were received in the laboratory in excellent condition. The A.O.A.C. method was carefully followed in obtaining the freezing-point depressions shown in Table 2. In practically every instance the

TABLE 2.—*Freezing-point depression of milk*

LAB. NO.	SOURCE	FAT	SOLIDS NOT FAT	FREEZING-POINT DEPRESSION	MILK FROM—
		per cent		°C.	
284	Sacramento	4.8	9.66	0.547	—
345	Salida	4.5	9.12	0.536	6 cows
346	Salida	4.78	9.80	0.537	6 cows
347	Salida	4.75	9.27	0.536	24 cows
348	Fresno	5.4	9.11	0.541	Guernsey cows
351	Fresno	5.9	9.68	0.541	Guernsey cows
354	Fresno	5.05	9.53	0.541	Guernsey cows
357	Fresno	5.35	9.32	0.541	Guernsey cows
384	Eureka	4.0	9.56	0.536	Mixed breeds
385	Eureka	5.35	9.29	0.534	Guernsey cows
434	Redding	3.4	8.50	0.537	—
436	Redding	4.4	8.60	0.536	—
448	Anderson	5.1	9.18	0.537	—
455	Sacramento	4.5	9.30	0.540	- -
459	Yuba City	4.95	9.29	0.536	—
487	Artesia	3.6	8.59	0.532	210 Holstein, 75 Guernsey, 75 Jersey
488	Downey	4.5	8.77	0.533	240 Holstein, 180 Guernsey, 60 Jersey
489	Hynes	4.7	10.19	0.547	120 Holstein, 15 Guernsey, 15 Jersey
490	Hynes	4.9	8.74	0.537	100 Holstein, 25 Guernsey, 25 Jersey
541	Eureka	4.4	8.87	0.529	Mixed breeds
542	Eureka	5.4	9.11	0.529	Guernsey cows
615	Sacramento	5.4	9.00	0.530	—
616	Sacramento	4.5	9.88	0.530	—
1148	Lakeport	—	—	0.536	—
			Average	0.536	

results are the average of closely agreeing replicate determinations obtained independently by two individuals. The thermometer of the instrument was carefully standardized according to the A.O.A.C. procedure. As a further precaution, it was also calibrated by the U. S. Bureau of Standards, which furnished a certificate for the instrument showing calibration data practically identical with those obtained here.

A survey of the data obtained at this Laboratory and of other recently published data would indicate that the accepted average freezing-point depression of 0.550° is somewhat too high. This view-point has been ac-

cepted by the authorities of New South Wales,[1] who as a result of a survey of genuine samples found mixed milk occasionally to have a freezing-point depression of 0.535° C. They therefore considered it advisable to alter the freezing point standard for milk from −0.550° to −0.535° C. In Western Australia,[2] a similar action has been taken. There the standard for the freezing-point depression is set at not less than 0.540° C.

It is of interest at this time to point out that in Bailey's data on 130 samples of authentic milk from individual cows 29 (22.3 per cent) had a freezing-point depression of 0.540° C. or less, and that out of 37 samples of milk from herds, 22 (57.9 per cent) had a freezing-point depression of 0.540° C. or less. The data obtained by Hortvet, Bailey, and Stubbs and Elsdon are combined in Table 3.

TABLE 3.—*Freezing-point depressions and frequencies*

FREEZING-POINT DEPRESSIONS	FREQUENCIES
0.523	6
0.529–.530	12
0.531–.532	14
0.533–.534	33
0.535–.536	53
0.537–.538	79
0.539–.540	137
0.541–.542	155
0.543–.544	172
0.545–.546	148
0.547–.548	123
0.549–.550	105
0.551–.552	70
0.553–.554	44
0.555–.556	25
0.557–.558	10
0.559–.560	16
0.561–.563	15
0.564–.566	7
Average 0.544	Total 1224

These data were examined statistically, but the results are virtually the same as those obtained by mere inspection of the table. The distribution curve is normal and very symmetrical. The mean is 0.544, the median 0.543.

It will be noted in Table 3 that out of 1224 examinations, 661 (54 per cent) show a freezing-point depression of 0.540 or less, and 1037 (84.7 per cent) show a depression of 0.550 or less. Inasmuch as it has been proved that the season of the year or feed does not affect the freezing-point de-

[1] *Analyst*, 62, 610 (1937).
[2] *Ibid.*, 63, 890 (1938).

pression of milk from healthy cows, there is no reason to assume that these values are not characteristic of much of the milk produced. The writer believes it advisable, therefore, in cases where control samples are not available, that a freezing-point standard of 0.540° C. be accepted for pure milk, especially when the results are to be used for the detection and quantitative estimation of added water.

ACKNOWLEDGMENT

A number of the freezing-point determinations reported in this paper were made by John H. Brandon, of the Dairy Service Laboratory.

CONCLUSION

An examination of published data and of the new data presented indicates that 0.540° C. is a more desirable figure than 0.550° C. for the average freezing-point depression of pure milk.

ESTIMATION OF PSEUDO-CUMIDINE IN ACID DYES

By C. F. Jablonski (U. S. Food and Drug
Administration, New York, N. Y.)

The Federal Food, Drug, and Cosmetic Act of 1938 makes mandatory the certification of all batches of coal-tar colors intended for use in foods, drugs, and cosmetics. Regulations governing the procedure for such certification and giving specifications for the purity of permitted coal-tar colors have been published by the Secretary of Agriculture.[1]

In these regulations there appears for the color FD&C Red No. 1, also known as Ponceau 3R, the specification: "Pseudo-cumidine, not more than 0.2 per cent." Pseudo-cumidine is one of the intermediates used in the manufacture of FD&C Red No. 1, and the following method is proposed for the estimation of small quantities of this intermediate, if present in uncombined form in the color. The method can also be used for the estimation of pseudo-cumidine in other acid dyes.

REAGENTS

(a) *Sodium hydroxide.*—Approximately 10%. Dissolve 10 grams of NaOH in 100 cc. of water.

(b) *Dilute sodium hydroxide.*—Approximately 0.125 N. Dissolve 1 gram of NaOH in 200 cc. of water.

(c) *Sulfuric acid.*—Approximately 1 N. Add 28 cc. of concentrated H_2SO_4 to 900 cc. of water. Cool, and dilute to 1 liter.

(d) *Sodium nitrite.*—10%. Dissolve 1 gram of $NaNO_2$ in 10 cc. of water.

(e) *Schaeffer's salt.*—1%. Dissolve 1 gram of beta-naphthol-6-sulfonate in 100 cc. of water.

(f) *Sodium carbonate.*—Approximately 2 N. Dissolve 110 grams of the anhydrous salt in 1 liter of water.

[1] *Fed. Reg.*, **4**, 1935 (1939).

To 10.0 grams of the sample add 200 cc. of water and heat carefully on the steam bath until dissolved. When cool, transfer to a 250 cc. separatory funnel and add 10 cc. of the 10% NaOH. Add 40 cc. of CHCl₃ and shake vigorously for about 5 minutes. After separation, draw off lower layer into another funnel.

Re-extract the dye solution three times more with CHCl₃, using successively 30, 20, and 10 cc., and shaking vigorously each time. Discard the aqueous color solution. Wash the combined CHCl₃ extracts five times with 40 cc. each of 0.125 N NaOH and finally with 25 cc. portions of water until the washings are colorless (usually 3–4 washings).

Transfer the CHCl₃ extract to a 200 cc. beaker, add 75 cc. of 1 N H₂SO₄, mix, and evaporate carefully over a tepid water bath, using a current of air to aid volatilization. After removal of the CHCl₃ add sufficient water to make approximately 100 cc., place the beaker in an ice bath, and cool its contents to 3–5° C. Add 0.1 cc. of NaNO₂ solution and keep reaction mixture cold for about 1 hour.

In a 500 cc. Erlenmeyer flask place 5 cc. of Schaeffer's salt solution and 45 cc. of Na₂CO₃ solution. Pour the diazo mixture slowly into the alkaline Schaeffer's salt solution, mixing thoroughly. Place the Erlenmeyer flask with the red color solution on the steam bath, maintaining a temperature of about 70° C. for 1 hour for complete color development. (The intensity of the color is proportional to the pseudo-cumidine present.)

Cool the color solution and add 15 cc. of strong HCl. In each of three (or more if necessary) 250 cc. separatory funnels place 50 cc. of amyl alcohol. Transfer about 40 cc. of the color solution to the first funnel and extract. Draw off the lower (aqueous) layer into funnel No. 2 and repeat the extraction, transferring the aqueous layer to funnel No. 3 and so on, until all the red color is extracted. Discard the aqueous solution. Repeat in 40 cc. aliquots with the balance of the color solution, passing each aliquot from funnel to funnel in the same order and discarding them, as soon as the color is extracted.

Wash the amyl alcohol extracts successively with 40 cc. portions of water, passing washings from one funnel to another in reverse order to that used during the extraction until the water extract is colorless.

Dilute the amyl alcohol in each funnel with an equal volume of gasoline or ligroin and extract the coloring matter successively with 100 cc. portions of water, following the order used above for washing. Repeat the extractions until all the color is removed from each solvent mixture. Evaporate the combined aqueous solutions in a casserole over a live steam bath. Dissolve residue with 150 cc. of hot water, transfer to a 300 cc. Erlenmeyer flask, add 10.0 grams of Na bitartrate and titrate hot with standard TiCl₃ in presence of a current of CO₂.

1 cc. of 0.1 N TiCl₃ = 0.00338 gram of pseudo-cumidine.

A 10.0 gram sample of certified Ponceau 3R treated in the manner described gave 0.00067 gram or = 0.0067 per cent pseudo-cumidine. To another 10.0 gram aliquot of the same sample 0.010 gram of pseudo-cumidine was added before the treatment. The total amount of cumidine recovered was 0.00977 gram. Subtracting the blank (0.00067) the net recovery was 0.00911 gram, or 91.1 per cent.

EFFECT OF LIGHT ON ERYTHROSINE AND BROMO ACID

By O. L. EVENSON (Cosmetic Division, U. S. Food and
Drug Administration, Washington, D. C.)

Erythrosine, the sodium salt of tetraiodofluorescein (Colour Index No. 773),[1] is one of the coal-tar colors that may be certified for use in coloring foods, drugs, and cosmetics in the United States. The color is listed[2] under the name FD&C Red No. 3 and to be certifiable it must meet the following specifications:

FD&C RED NO. 3

SPECIFICATIONS

Disodium salt of 9-o-carboxyphenyl-6-hydroxy-2, 4, 5, 7-tetraiodo-3-isoxanthone.

Volatile matter (at 135° C.), not more than 12.0%.

Water-insoluble matter, not more than 0.2%.

Ether extracts, not more than 0.1%.

Chlorides and sulfates of sodium, not more than 2.0%.

Sodium carbonate, not more than 0.5%.

Sodium iodide, not more than 0.4%.

Mixed oxides, not more than 1.0%.

Permitted range of organically combined iodine in pure dye, free from water of crystallization, 56.8–58.5%.

Pure dye (determined gravimetrically), not less than 85.0%.

Similarly bromo acid (tetrabromofluorescein) may be certified as D&C Red No. 21[2] for use in drugs and cosmetics, provided it meets the following specifications:

D&C RED NO. 21

SPECIFICATIONS

2, 4, 5, 7-tetrabromo-3, 6-fluorandiol.

Volatile matter (at 135° C.), not more than 6.0 %.

Insoluble matter (alkaline solution), not more than 1.0%.

Ether extracts (from alkaline solution), not more than 0.5%.

Chlorides and sulfates of sodium, not more than 2.0%.

Mixed oxides, not more than 1.0%.

Free bromine, not more than 0.02%.

Permitted range of organically combined bromine in pure dye, 47.5–51.5%.

Pure dye (determined gravimetrically), not less than 93.0%.

Several investigations have been made of the effect of light on dyes of the erythrosine-bromo acid type. Tappeiner and Raab[3] found that certain low forms of life were killed by exposure to light in the presence of eosin, the sodium salt of bromo acid. They attributed this to fluorescence and called substances exhibiting such effects, "Photodynamic." Dreyer[4] found that as silver bromide plates can be sensitized with erythrosine to the

[1] Colour Index of the Society of Dyers and Colourists, England.
[2] *Fed. Reg.*, **4**, 1936 (1939).
[3] *Münch med. Wochschr.*, **47**, 5–7 (1900).
[4] *Proc. Royal Acad. Sci., Copenhagen*, No. 3, 393–97 (1903).

yellow and green rays of the spectrum, so can bacteria be sensitized to the same rays with this color. Neisser and Halberstadter[1] showed that bacteria were killed when sensitized with erythrosine and exposed to light. These investigators mentioned that iodine might be released from the erythrosine under the influence of light but concluded that therapeutic effects were due to the dye sensitizing the cells to the more deeply penetrating yellow and green rays rather than to the action of iodine. Jodlbaur and Tappeiner[2] postulated oxygen as the causative agent, since their experiments seemed to show that benzoic acid was produced from benzyl alcohol and that indigo was oxidized in the presence of erythrosine exposed to light. They also reported that exposed solutions of eosin and erythrosine developed acidity.

The following investigation of the influence of light on erythrosine and bromo acid was made incidental to determining the possibility of estimating these colors by a spectrophotometer. Certified colors were used and sodium iodide was determined by the procedure given in *Methods of Analysis*, of the Association of Official Agricultural Chemists, 1935, page 259. The extent of the fading of erythrosine was measured with a spectrophotometer.

From a freshly made aqueous solution of erythrosine (FD&C Red No. 3), containing 1 per cent of pure dye, a dilute aqueous solution containing 50 mg. of dye per liter was prepared; 20 cc. aliquots of this dilute solution (equal to 1 mg. of dye) were placed in each of four 100 cc. volumetric flasks. To two of these flasks 50 cc. portions of 95 per cent ethyl alcohol were added. Finally all four flasks were made to volume with water. These solutions were then exposed to direct sunlight for periods of 10 and 20 minutes. The Bunsen extinction coefficient ($E = -\log_{10}$ transmittancy) at 530 mμ was determined before and after exposure. The results are found in Table 1. Each of these results is the average of two closely agreeing determinations. It is evident from Table 1 that erythrosine fades rapidly in water solution, whereas in 47.5 per cent alcohol the rate of fading is much slower.

TABLE 1.—*Effect of exposure of erythrosine solutions to sunlight*

CONCENTRATION	SOLVENT	EXPOSURE	E* AT 530 mμ	FADING
per cent		*minutes*		*per cent*
0.001	H$_2$O	0	.96	
0.001	H$_2$O	10	.77	20
0.001	H$_2$O	20	.62	35
0.001	47.5% alcohol	0	1.08	
0.001	47.5% alcohol	10	1.08	0
0.001	47.5% alcohol	20	1.05	3

* E = $-\log_{10}$ transmittancy. Layer depth =1 cm.

[1] *Deut. med. Wochschr.*, **30**, 265–69 (1904).
[2] *Deut. Archiv. klin. Med.*, **82**, 520 (1904).

Experiments were then made on 1 per cent aqueous solutions of erythrosine, placed in 100 cc. volumetric flasks and exposed to the sun for periods of several months. At the end of the exposure an appreciable quantity of the characteristic color acid of erythrosine had precipitated. Sodium iodide was determined before and after exposure. Table 2 gives the results.

TABLE 2.—*Increase of sodium iodide in solutions of erythrosine after exposure to light*

NO.	SOLVENT	CONCENTRATION OF COLOR	TOTAL NaI IN SOLUTION	
			BEFORE EXPOSURE	AFTER EXPOSURE*
		per cent	*mg.*	*mg.*
1	H_2O	1	3	68
2	H_2O	1	3	125

* Time of exposure: No. 1—3½ months (Dec. 14, 1934 to April 2, 1935);
No. 2—6 months (May 1, 1934 to November 1, 1934).

Since the sodium iodide content increases in the exposed samples, it seems that the erythrosine molecule loses iodine under the influence of light. The presence of color acid in the exposed solutions further shows that the solutions developed acidity. The following reaction mechanism may explain this. Under the influence of light, iodine organically combined in the dye is split off. The iodine reacts with the slight excess of sodium carbonate that is always present in these colors. This results in the formation of carbon dioxide, which precipitates the color acid. A formation of hydriodic acid and iodic acid may be postulated as intermediary steps.

In the presence of erythrosine other dyes in aqueous solution also appear to be destroyed by the action of light. Table 3 shows the case of mixtures of erythrosine and sodium indigo disulfonate (also known to the trade as "indigotine" and certifiable as FD&C Blue No. 2). Mixtures of erythrosine and indigotine in 250 cc. volumetric flasks were exposed for about 3 hours to direct sunlight. The Bunsen extinction coefficients were determined at or near the two points of maximum absorption, 610 mμ for indigotine and 530 mμ for erythrosine. The percentage of indigotine destroyed, as computed from the reduction in the value of E, is shown in Table 3.

Table 3 indicates that the presence of as little erythrosine as 0.1 mg. per liter may have an appreciable effect on indigotine when the mixture is exposed to light. In mixture No. 4 the presence of 2 mg. of erythrosine caused a destruction of 14 mg., or 70 per cent of the indigotine present. Similar experiments on other water-soluble certifiable food colors showed that all except fast green (FD&C Green No. 3), brilliant blue (FD&C Blue No. 1), and tartrazine (FD&C Yellow No. 5) were destroyed to some

degree by direct sunlight in the presence and as a result of decomposition of erythrosine. The three colors mentioned appear to be quite stable, but the possibility remains that they, too, may be affected by a more prolonged exposure. The observation that certain coal-tar colors in the presence of erythrosine undergo decomposition and destruction in sunlight may be of practical significance, since erythrosine is used in food color mixtures.

TABLE 3.—*Effect of light on aqueous solutions of mixtures of indigotine and erythrosine*

| TEST NO. | DYE | CONCENTRATION | E* | | | APPROXIMATE AMOUNT DESTROYED |
			MEASURED AT WAVE LENGTH	BEFORE EXPOSURE	AFTER EXPOSURE†	
		mg./l.	mμ			per cent
1	Indigotine alone	20	610	0.88	0.88	0
			530	.18	.18	
2	Indigotine plus erythrosine	20	610	.88	.83	6
		0.1	530	.18	.18	
3	Indigotine plus erythrosine	20	610	.88	.52	40
		1	530	.28	.13	
4	Indigotine plus erythrosine	20	610	.88	.28	70
		2	530	.38	.06	
5	Indigotine plus erythrosine	20	610	.88	0	100
		20	530	2.20	.06	

* Layer depth 1 cm.
† Exposed 3 hours in 250 cc. volumetric flasks.

In the light of the experiments described it seems reasonable to assume that the fading of erythrosine is due to a release of halogen by the action of sunlight, resulting in the formation of a colorless compound. Experiments made in this laboratory also show that eosin (C.I. No. 768), fades rapidly on exposure to sunlight.

That bromo acid, the color acid of eosin, releases bromine in the presence of sunlight was shown in the following way. Dry bromo acid was placed in two 500 cc. Erlenmeyer flasks. A strip of starch iodide paper, held in place by a rubber stopper, was suspended in the neck of each flask as well as in the neck of a third flask, designated the blank and containing no bromo acid. The neck of each flask was covered with a stiff paper to protect the starch iodide paper from the direct rays of the sun. The blank and one of the flasks containing bromo acid were then placed in direct

sunlight for 10 hours. The remaining flask was placed in the dark. After exposure the starch iodide paper in the flask exposed to light and containing bromo acid turned blue when moistened with water, while the papers in the other flasks remained colorless. This experiment was repeated twice, and in both cases the results were the same. The release of free halogen from the dye as a result of the action of sunlight therefore seems to be definitely indicated. Since bromo acid is certifiable for use in drugs and cosmetics, the role of the released halogen should not be overlooked in considering the therapeutic, allergic, or antiseptic action of the color.

SUMMARY

Aqueous solutions of erythrosine fade rapidly when exposed to direct sunlight, while the fading in 47.5 per cent alcohol is much slower. Iodine appears to be liberated from the erythrosine molecule and bromine from bromo acid by the action of light. Certain other dyes, in the presence of erythrosine, are destroyed, as a result of the action of light on the erythrosine present.

THE DETERMINATION OF QUINALDINE IN CERTAIN COAL-TAR COLORS

By W. H. KING (Cosmetic Division,* U. S. Food and Drug Administration, Washington, D. C.)

Quinaldine (2-methyl quinoline) is an intermediate used in the manufacture of D&C Yellow No. 10 (Quinoline Yellow W.S.) and D&C Yellow No. 11 (Quinoline Yellow S.S.). Batches of these colors are acceptable for certification by the U. S. Department of Agriculture for use in drugs and cosmetics, provided they conform to certain specifications.[1] Among these specifications is one reading: "Quinaldine, not more than 0.2 per cent."

The following method is proposed for the determination of quinaldine in D&C Yellow Nos. 10 and 11. It is based on the separation of quinaldine from the colors by steam distillation, followed by extraction from the concentrated steam distillate and precipitation from acid solution as a salt of phosphotungstic acid, which salt may be weighed before and after incineration. The reactions involved are:

$$3C_{10}H_9N + P_2O_5 \cdot 24WO_3 \cdot xH_2O \rightarrow$$
$$\rightarrow (C_{10}H_9N)_3PO_4 \cdot 12WO_3 \rightarrow HPO_3 \cdot 12WO_3.$$
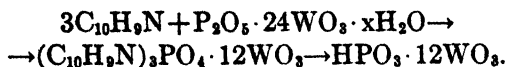
The test appears to be sensitive, 1–2 mg. of quinaldine in 100 cc. giving a distinct precipitate with phosphotungstic acid.

METHOD

Place 25 grams of the color in a 2 liter flask equipped for steam distillation and having all glass connections. Add an excess of Na_2CO_3 (usually about 10 grams) in 50 cc. of hot water and steam distil. In the case of D&C Yellow No. 11 pass the steam through at a rather rapid rate to stir the insoluble dye during the distillation. Collect the distillate in a 1500 cc. beaker containing 1–2 cc. of concentrated HCl. Continue the distillation until all the quinaldine has come over (approximately 1000 cc.). Toward the end of the distillation drain the cooling water from the condenser and allow steam to pass through for several minutes. At the finish the liquid in the distilling flask must be alkaline to phenolphthalein (if not, repeat using more Na_2CO_3) and the distillate acid to congo red paper. Concentrate the distillate to approximately 100 cc. by boiling. If ammonium salts are present in the original sample, it is necesary to purify the quinaldine as directed under (*b*). Otherwise continue as directed under (*a*).

(*a*) Transfer the concentrated distillate to a 250 or 300 cc. beaker and add dropwise, with stirring, an excess of a 10 per cent filtered solution of phosphotungstic acid (about 1.5 cc. for each 10 mg. of quinaldine expected). Heat almost to boiling, let stand in a warm place until the precipitate has coagulated, and filter with suction on an ignited and weighed Gooch crucible. Dry to constant weight at 135° C. Cool in a desiccator and weigh as soon as cooled. The weight of dry salt multiplied by 0.131 gives the weight of quinaldine in the sample.

Ignite the contents of the crucible to constant weight at 500–550° C., cool as before, and weigh. The weight of the ash multiplied by 0.152 gives the weight of quinaldine in the sample.

(*b*) Transfer the concentrated distillate to a separatory funnel, neutralize with Na_2CO_3, adding 0.5–1.0 gram in excess, and extract with two 50 cc. portions of $CHCl_3$, washing each portion with 1–2 cc. of water in a second separatory funnel. Filter each portion through a small pledget of cotton into a 300 cc. tall-form beaker. Add to the combined $CHCl_3$ extracts 1 cc. of concentrated HCl and 100 cc. of water and boil off the $CHCl_3$. Proceed as directed under (*a*), beginning with the addition of the phosphotungstic acid.

From the weights W_1 and W_2 of $Q_3PO_4 \cdot 12WO_3$ and $HPO_3 \cdot 12WO_3$, respectively, calculate the molecular weight of quinaldine, Q. This gives a check on the identity of the amine. The formula,

$$Q = \frac{2864(W_1 - W_2)}{3W_2} - 5,$$

may be derived as follows:

If Q represents one molecule of quinaldine, the change taking place when W_1 grams of the salt, $Q_3PO_4 \cdot 12WO_3$, is ignited to give W_2 grams of the ash, $HPO_3 \cdot 12WO_3$, may be represented by—

(1) $Q_3PO_4 \cdot 12WO_3 - HPO_3 \cdot 12WO_3 = 3Q + O - H$; and

(2) $W_1 - W_2 = \Delta W.$

If 1 mol of the salt is involved, 1 mol of the ash will be formed and the loss in weight on ignition, $(\Delta W)_m$, will be—

(3) $(\Delta W)_m = 3Q + O - H = 3Q + 16.0 - 1.0 = (3Q + 15)$ grams.

For X mols of salt, weighing W_1 grams, there would be formed X mols of ash, weighing W_2 grams, and the loss in weight, ΔW, would be—

(4) $\Delta W = X(\Delta W)_m = X(3Q + 15)$ grams.

Further, the relation between X, W_2, and the molecular weight of the ash, M.W., is—

$$\frac{\text{Actual weight of ash, } W_2}{\text{Molecular weight of ash, M.W.}} = \text{Number of mols, } X,$$

where M.W. is the molecular weight of $HPO_3 \cdot 12WO_3$, or 2864.0. This gives—

(5)
$$X = \frac{W_2}{2864}.$$

A combination of equations (2), (4), and (5) gives—

(6)
$$\Delta W = W_1 - W_2 = \frac{W_2(3Q + 15)}{2864};$$

or

(7)
$$Q = \frac{2864(W_1 - W_2)}{3W_2} - 5.$$

When minute quantities of quinaldine are determined, i.e. $(W_1 - W_2)$ is small, equation (7) can, of course, only be depended upon to give a very rough estimate of Q.

DISCUSSION

Kahane and Kahane[1] have shown that with phosphotungstic acid certain simple organic bases form salts that have the general formula: $B_3(PO_4 \cdot 12WO_3)$. These compounds, upon ignition at dull red heat, are converted into the compound: $HPO_3 \cdot 12WO_3$.

According to these formulas, the factors for conversion to quinaldine (Q) would be—

$$0.130 \times Q_3(PO_4 \cdot 12WO_3) = Q;$$

and

$$0.150 \times HPO_3 \cdot 12WO_3 = Q.$$

The factors recommended in the method differ slightly, since they were determined experimentally, and take into consideration the solubility of quinaldine phosphotungstate. For the determination of these factors, Eastman's redistilled quinaldine, white label (216), was used in concentrations ranging from 5 to 200 mg. per 100 cc. Table 1 shows the results.

Several phosphotungstic acids are known,[2,3] the ratio, $P_2O_5 : WO_3$, varying from 1:7 to 1:24. The compound most commonly referred to as "phosphotungstic acid" seems to be $P_2O_5 \cdot 24WO_3 \cdot xH_2O$. Barnes and Peters,[4] however, claim that commercial phosphotungstic acid is the "1:24 acid," containing some "1:18 acid."

[1] *Bull. Soc. Chem.*, **3**, 621 (1936).
[2] Roscoe and Schorlemmer, *Treatise on Chemistry*, Vol. II, p. 1067. McMillan & Co., Ltd. (1907).
[3] Mellor, A comprehensive *Treatise on Inorganic and Theoretical Chemistry*, Vol. XI, p. 863. Longmans, Green & Co. (1931).
[4] *Biochem. J.*, **26**, 2208 (1932).

TABLE 1.—*Factors obtained by precipitating various amounts of quinaldine with phosphotungstic acid*

QUINALDINE	FACTOR FOR DRY SALT	FACTOR FOR ASH
mg./100 cc.		
5	0.144*	0.169*
15	0.130	0.148
25	0.134	0.156
35	0.131	0.153
45	0.131	0.152
50	0.131	0.152
55	0.131	0.151
70	0.132	0.152
90	0.132	0.151
100	0.129	0.149
200	0.130	0.152
	Av. 0.131	Av. 0.152

* Not included in average.

In order to determine possible errors in the factors, due to variation in the composition of the phosphotungstic acid, experiments were made on eight commercial samples of the acid representing old and recent output of seven manufacturers; 50 mg. of quinaldine was used as a precipitant. The results are shown in Table 2.

TABLE 2.—*Factors obtained by precipitation of 50 mg. of quinaldine with various brands of phosphotungstic acid*

BRAND OF PHOSPHOTUNGSTIC ACID USED	FACTOR FOR DRY SALT	FACTOR FOR ASH
A	0.133	0.154
B (old)	0.131	0.152
B (new)	0.131	0.153
C	0.130	0.150
D	0.132	0.153
E	0.132	0.151
F	0.131	0.152
G	0.132	0.155
	Av. 0.131	Av. 0.152

While these results (Table 2) indicate a remarkable uniformity of commercial lots of phosphotungstic acid, it seems advisable that each analyst check his own batch of the acid before accepting the above-recommended factors.

Finally, known quantities of quinaldine were added to samples of D&C Yellow No. 10 and D&C Yellow No. 11, which samples, when examined by the proposed method, had not shown the presence of free quinaldine.

Recoveries ranged from 96 to 99 per cent, the main error being insufficient recoveries when less than 1000 cc. of distillate was collected. Incidentally, the fact that the samples prior to the addition of quinaldine failed to give positive results indicates that the isolation treatment does not decompose these colors.

DETERMINATION OF HYDROCYANIC ACID BY THE PICRIC ACID METHOD AND THE KWSZ PHOTOMETER

By J. T. SULLIVAN (U. S. Regional Pasture Research Laboratory, State College, Pa.*)

The picric acid test for hydrocyanic acid known as the Guignard test[1] is commonly used for qualitative, but less often for quantitative purposes. Some recent applications of the test for quantitative purposes have been made. Rogers and Frykholm[2] divided white clover plants into five groups according to the intensity of color of the test papers. Boyd, Aamodt, Bohstedt, and Truog[3] described a method that involves the heating of an alkaline picrate solution with the distillate of Sudan grass and a visual comparison of the color change with standards. In a private communication, F. T. Boyd of the Everglades Experiment Station, Florida, furnished more details of the method. B. W. Doak,[4] in a footnote to a recent paper, endorsed the Boyd procedure for white clover.

The procedure described here is an adaptation of the Boyd method to the determination of hydrocyanic acid in individual plants of white clover. Of all the plants studied 70 per cent contained .001 per cent or less of hydrocyanic acid on the fresh weight basis. Owing to the limited quantity of material available, a determination of less than .05 mg. of hydrocyanic acid was often necessary. Such small quantities could be more accurately determined by the KWSZ photometer than by visual comparison with standards.

THE METHOD

REAGENTS

(a) *Alkaline picrate solution.*—Dissolve 25 grams of anhydrous Na_2CO_3 and 5 grams (corrected for moisture content) of picric acid in 1000 cc. of water.

(b) *Potassium hydroxide.*—2% solution.

(c) *Toluene.*

(d) *Copper sulfate.*—10 grams of $CuSO_4 \cdot 5H_2O$ and 1 drop of H_2SO_4 dissolved in water and diluted to 100 cc.

(e) *Potassium cyanide.*

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[1] *Compt. rend.*, **142**, 545–53 (1906).
[2] *J. Agr. Res.*, **55**, 533–37 (1937).
[3] *J. Am. Soc. Agron.*, **30**, 569–82 (1938).
[4] *New Zealand J. Sci. Tech.*, **10**, 3A, 163–66 (1938).

Place 10 grams of fresh white clover leaves in a 500 cc. short-necked Kjeldahl flask and add 5 cc. of toluene. Close the flask tightly with a rubber stopper, and allow to stand several days at room temperature. Remove the stopper, immediately make connection to a distilling apparatus, and steam distil from the same Kjeldahl flask. Distil 80–90 cc., catching the distillate in a 100 cc. beaker containing 5 cc. of the KOH. During the first half of the distillation have the tip of the condenser dipping below the surface of the liquid in the beaker. Transfer the distillate into a 100 cc. volumetric flask and dilute to the mark with water, mean-

FIGURE 1

CHANGE OF PERCENTAGE
TRANSMISSION WITH CONCN. OF HCN

PERCENTAGE TRANSMISSION

MILLIGRAMS OF HCN
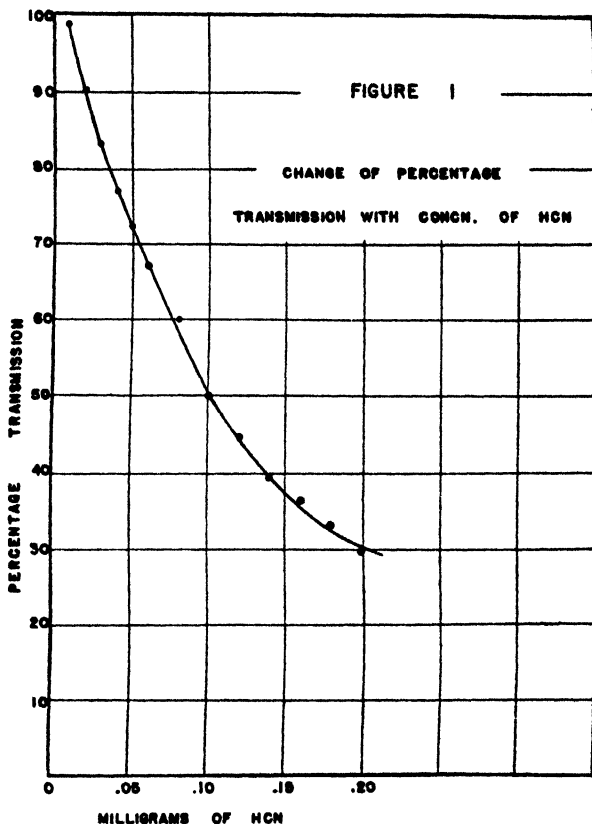
while decanting and discarding the supernatant toluene. Pipet 20 cc. or less of the distillate and 10 cc. of the alkaline picrate solution into a 25×150 mm. test tube. (If less than 20 cc. of distillate is used, add water to a total volume of 30 cc.) Plug the test tube loosely with cotton and stand it upright in boiling water for exactly 5 minutes; remove, and cool to room temperature. With each set of determinations prepare a blank by heating 10 cc. of the alkaline picrate solution with 20 cc. of water or with the same volume of 0.1 % KOH solution. Without diluting or rinsing, pour the entire contents of a test tube into one of the two absorption cells of the photometer. Balance the instrument against the blank, using the $CuSO_4$ solution as a light filter, and read the transmission of the unknown in the other absorption cell. From the curve prepared from standards, convert the percentage transmission into weight of HCN.

The standardization curve was prepared as follows: 1.015 grams of KCN (Baker's analyzed, assay 95.0%) was dissolved in 1 liter of water to give a solution containing 0.4 mg. of HCN per cc. Its strength was checked by alkaline titration against 0.02 N AgNO$_3$. A dilution was then prepared with 9 parts of water to give a solution containing 0.04 mg. of HCN per cc. A series of standards was made from this solution by diluting to 100 cc. aliquots varying from 1.25 to 25.0 cc.; 20 cc. fractions of these standards, containing 0.01–0.20 mg. of HCN, were heated as before with the alkaline picrate solution. The percentage transmissions were determined and plotted against the amount of HCN present. The curve shown in Figure 1 was drawn freely through the points obtained.

DISCUSSION

The conditions under which hydrocyanic acid is liberated from its parent glucoside and distilled need more exact definition. Immediate distillation of a sample with chloroform gave in some cases as high results as could be obtained with that particular sample, but in most cases higher results were obtained if an autolysis period of 24–48 hours intervened before the distillation. The same was true with toluene, but the latter, being lighter than water, had the advantage of not interfering with the making of the distillate to volume. An autolysis of several days without any preservative, or freezing and thawing followed by distillation with or without preservation, gave variable results. Autolysis for one or two days at room temperature with toluene always gave as high or a higher yield of hydrocyanic acid than any other treatment of a duplicate sample. Grinding of the fresh sample was omitted, but it would undoubtedly influence the time necessary for autolysis. The addition of water to the sample did not appear necessary, and its omission hastened the steam distillation. Immediate heating of the distillate with the alkaline picrate is advisable, but the reading of the results in the photometer may be delayed a day or two.

Because of the deep color of the picric acid reagent little difference in light transmission between samples containing low and high amounts of hydrocyanic acid was shown when the photometer was balanced against water, either without a filter or when a 450 or a 650 mμ glass filter was used. The curve found most useful (Figure 1) was obtained by using a copper sulfate solution in the water bottles of the photometer as the only light filter and balancing the instrument against a blank determination containing the alkaline picrate reagent.

The range of variation of percentage transmission in triplicate determinations of the same distillate averaged 0.4 over the whole curve; that of duplicate distillations was greater, being about 3.0 when 0.1 mg. of hydrocyanic acid was present. There is a greater sensitivity at lower concentrations. It is not advisable to use aliquots containing more than 0.2 mg. of hydrocyanic acid.

In order to study the agreement with the alkaline titration method[1] it was necessary to use larger samples than are usually available and to use large dilutions for the picric acid test. The results, which follow, average 8 per cent higher than those by the titration method.

	PICRIC ACID METHOD			TITRATION		
	ALIQUOT OF DISTILLATE	ACTUAL HCN DETERMINED	TOTAL HCN	ALIQUOT OF DISTILLATE	ACTUAL HCN DETERMINED	TOTAL HCN
	mg.	*mg.*			*mg.*	*mg.*
1	1/20	.140	2.8	4/5	2.00	2.50
2	1/100	.055	5.5	3/4	3.75	5.00
3	1/100	.059	5.9	4/5	4.27	5.34
4	1/100	.0325	3.25	4/5	2.61	3.27

SUMMARY

A procedure is proposed for the determination of small quantities of hydrocyanic acid in individual white clover plants. It utilizes the KWSZ photometer for the measurement of the color change in the alkaline picrate—hydrocyanic acid reaction. Hydrocyanic acid may be measured in amounts of 0.01–0.20 mg. The various steps of the procedure, especially the conditions of autolysis and distillation, need further study, particularly if the method is applied to other plants than white clover.

DETERMINATION OF 1,4 DIHYDROXY-ANTHRAQUINONE IN D&C GREEN NO. 5

(Alizarine Cyanine Green F)

By G. R. CLARK (Cosmetic Division,* U. S. Food and Drug Administration, Washington, D. C.)

The regulations[2] governing listing and certification of coal-tar colors under the Federal Food, Drug, and Cosmetic Act of 1938 limit the amount of the intermediate 1,4 dihydroxy-anthraquinone permitted in the color D&C Green No. 5 to not more than 0.2 per cent.

The colorimetric method presented here is suggested for the determination of 1,4 dihydroxy-anthraquinone. The intermediate is separated by continuous extraction with diethyl ether from a slightly acid solution of D&C Green No. 5 and is in turn removed from the ether by a 5 per cent sodium hydroxide solution, with which reagent it forms a characteristic reddish-blue color.

Eastman's 1,4 dihydroxy-anthraquinone, technical grade, was used for these experiments. The material was purified by precipitation with hydrochloric acid from an alkaline solution, followed by three crystallizations of the precipitate from alcohol.

[1] *This Journal*, 19, 94 (1936).
* D. Dahle in charge.
[2] *Fed. Reg.*, 4, 1935 (1939).

PROPOSED METHOD

REAGENT

Sodium hydroxide.—5%. Dissolve 5 grams of NaOH in 100 cc. of water.

1,4 *Dihydroxy-anthraquinone.*—A freshly prepared, solution containing 0.1 gram of 1,4 dihydroxy anthraquinone dissolved in 100 cc. of a 5% NaOH solution.

DETERMINATION

Dissolve 2 grams of sample in 20 cc. of the 5% NaOH solution, dilute to 100 cc. transfer to a continuous extractor, and add sufficient concentrated HCl to neutralize the NaOH and give 2–3 drops (not more) in excess. Make to a volume suitable for the extractor and extract with ether, testing 50 cc. portions of the extract from time to time by shaking in a small separatory funnel with a few cc. of the 5% NaOH reagent until the characteristic reddish-blue color fails to develop (complete extraction). Save the ether and also the NaOH solution used in testing for complete extraction. (four hours was found to be more than sufficient to extract 6 mg. from 300 cc. of sample solution.)

Evaporate the ether extracts, including the portions tested for complete extraction, to about 75 cc.; transfer to a separatory funnel, and extract with 25 cc. portions of the 5% NaOH reagent until no more color is extracted. (Some D&C Green No. 5 may remain in the ether layer.) Combine the NaOH extracts with the NaOH previously used in testing for complete ether extraction, and shake in a separatory funnel with 10–15 cc. portions of ether until all D&C Green No. 5 is removed.

Make the volume of the combined NaOH extracts to 250 cc. with the 5% NaOH reagent, mix, and pipet 100 cc. into a suitable comparison tube. Place about 98 cc. of the 5% NaOH reagent in another comparison tube, which serves as the standard. From a 10 cc. buret, graduated in 0.05 cc., add 0.1% solution of 1,4 dihydroxy-anthraquinone to the standard tube until the color of the standard matches the unknown. For the final color comparison dilute both solutions to the *same* volume with the 5% NaOH reagent. Calculate the amount of 1,4 dihydroxy-anthraquinone present as follows:

$$\frac{\text{cc. standard used} \times 0.25}{\text{sample weight}} = \% \text{ of 1,4 dihydroxy-anthraquinone present.}$$

DISCUSSION

A 2 gram sample of D&C Green No. 5 contained sufficient 1,4 dihydroxy-anthraquinone to require 0.8 cc. of the standard, equivalent to 2 mg., or 0.1 per cent; 4.5 mg. of 1,4 dihydroxy-anthraquinone was added to 2 grams of this dye, and the recovery was determined. The amount of standard required to match the color was 2.5 cc. This is equivalent to 6.25 mg., which, less the 2 mg. originally present, indicates a recovery of 4.25 mg. of the 4.5 mg. added, or 94 per cent.

BOOK REVIEW

Standard Chemical and Technical Dictionary. By H. Bennett. Chemical Publishing
Co., Inc., New York, N. Y. 1939. 638 pp. Price $10.00.

In these days of specialized scientific research and writing it would seem that
it is not possible to have too many sources of information to assist the searcher in
acquiring the necessary knowledge to treat his subject efficiently and thoroughly.

Therefore this publication will be welcomed by all scientists working in the field
covered. Its author asserts that it is "A condensed technical work book for students,
writers, technicians, engineers, scientists, and all others who need assistance in keep-
ing up with the many new chemical, physical, mathematical, and technical words
and expressions."

The work seems to be well done and the entries quite adequate. Only continued
practical use of a dictionary, however, can prove its worth. If on first trial the user
finds that his special subjects are treated to his satisfaction, he considers it a good
book; if, on the other hand, he expects the unusual and does not find it, he disap-
proves, or even condemns a publication before giving it a fair trial. Therefore the
writer can not be too critical when she did not find such entries as "rose bengale,"
'desoxycholic acid," and "Reinecke salt," and considers that not enough informa-
ion was given for the word "esculin."—Marian E. Lapp.

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